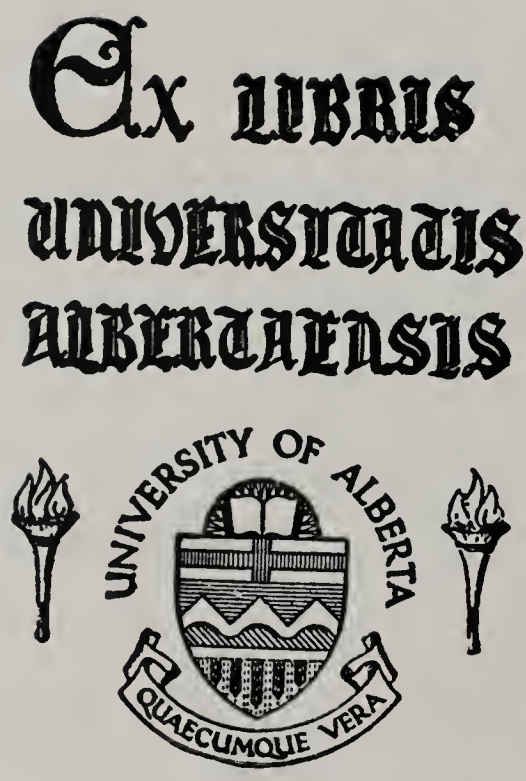

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THE DNA CONTENT OF CENTRIOLES OF NEUROSPORA CRASSA DURING DIVISIONS I AND IV OF ASCOSPOROGENESIS

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The DNA content of centrioles of Neurospora crassa during Divisions I and IV of ascosporogenesis" submitted by Brian Raymond McDonald in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The results of observations on Feulgen-stained preparations of developing asci of Neurospora crassa are presented. Photographic evidence presented shows that the centrioles during Divisions I and IV are Feulgen-positive and hence contain deoxyribonucleic acid (DNA). The presence of DNA in the centriole provides this extra-nuclear body with the necessary genetic information for its independent division prior to that of the nucleus. The occurrence of DNA in the centriole is discussed in the light of the centromere - centriole homology.

From observations of iron-haematoxylin stained preparations, it is evident that the centriole, with an internal spindle, divides prior to nuclear division.

A variation in size of the centriole was noted throughout the four divisions of ascosporogenesis.

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INTRODUCTION

The importance of the centrosome and especially the centriole in the process of nuclear division was already understood in the early days (1885) of cellular biology. Boveri (see Darlington, 14), at that time, noted that the centrosome was "the self-propagating body which during division in many organisms lies at the two poles of the spindle and appears to determine its orientation" (p. 573). Within the centrosome, a centriole can usually be found, the structure of which is defined as "a minute body, rod or granule . . . frequently considered to be the active, self-perpetuating, division center of the cell" (20). Undoubtedly, the early observation of the division of the extra-nuclear organelle led to the idea of its possible involvement in nuclear division. Cytology, however, is indebted to Cleveland (8, 9, 10, 11) for the details of this interaction. His precise and classical study dealing with the mode of centriole replication and movement has opened up new avenues for research. The characteristic of the centriole as a self-replicating body (its division takes place prior to that of the nucleus) might necessitate the occurrence of nucleic acids within the organelle. However, the results of searches for DNA in the centriole of animal cells have been negative. Mazia's recent review (26) with regard to the occurrence of DNA in centrioles is therefore carefully worded. He states that centrioles might "contain only a few replicating molecules," thereby indicating that the actual amount of nucleic acid (DNA) per centriole might not be detectable.

The recent discovery, however, that other extra-nuclear bodies such as mitochondria (34) and chloroplasts (7, 12) contained DNA, strengthened the expectation that the centriole might not be different in this respect. In the work reported on in this thesis, the centrioles of Neurospora crassa occurring during ascosporogenesis have therefore been examined for DNA content.

LITERATURE REVIEW

As early as 1897 Harper (18) described central bodies in the Ascomycete, Erysiphe. He later confirmed these observations in the mold, Phyllactinia (19). In both publications (18, 19) the term "central body" was chosen by Harper in preference to the term "centrosphere" or "centrosome" (19). In 1927 Dodge (15) described in Neurospora tetrasperma a sharply pointed, deeply-stained mass at each end of the spindle in metaphase I. Dodge called these deeply-stained masses centrosomes. In a later publication, however, Dodge, Singleton and Rolnick (16), only mentioned the probable occurrence of centrioles in N. tetrasperma. In addition, Singleton (36) published a description of the chromosome morphology and the chromosome cycle in the ascus of N. crassa which included microphotographs of centrioles. Some of the work reported in this publication includes earlier material by McClintock (27) on chromosome and centriole morphology. Somers et al., (37) observed in the vegetative cycle of N. crassa an eighth stainable body which they interpreted as a heterochromatic chromosome. On the other hand, Ward and Ciurysek (42) recognized such stainable bodies as centrosomes in their mitotic metaphase plates. Weijer et al., (46) favor a similar explanation. Recently, Weijer et al., (45) described centrioles in the somatic nuclei of N. crassa and Gelasinospora tetrasperma. Lu (24) also reported centrioles in N. crassa. Although the occurrence of centrosomes and centrioles have been reported in other Ascomycetes (a complete review can be

found in Olive (29)), the proof for the occurrence of these extra-nuclear bodies in the fungi was obtained by electron microscopy of the centriole of the Oomycete, Albugo candida (5). Lindegren et al., (23) recently published an electron microphotograph of the centriole of the Ascomycete, Saccharomyces. According to Cleveland (8, 9, 10, 11), the centriole can be regarded as an extra-nuclear body with the capacity for self-replication. As Weijer et al., (46) pointed out, the capacity for self-replication "might appear to qualify the centriole as a carrier of genetic factors and hence the question of the presence and amount of nucleic acid is crucial" (p. 156). This prediction is in line with the views of Mazia (26), who expected "the truly replicating 'germ' in (the) centriole to contain only a few replicating molecules, while the rest of the centriole need contain none" (p. 138). It also agrees with recent findings of the occurrence of DNA in other extra-nuclear bodies such as mitochondria (34) and chloroplasts (7, 12). The first report on the possible occurrence of DNA in fungal centrioles appeared in the literature when Weijer (43), using the Feulgen staining technique on Neurospora conidia and conidial germination tubes, described Feulgen-positive centrioles during karyokinesis (Juvenile cycle). Recently, Weijer et al., (46) published microphotographs of Feulgen-positive centrioles occurring during Maturation cycles I and II of N. crassa*. Schrader (35), using

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* It is of interest to note that Stich (39) has reported RNA in centrioles of Cyclops strenuus. Amano (1) found the staining of centrioles in vertebrate cells by iron-haematoxylin indicative of RNA, but cites earlier failures to demonstrate these extra-nuclear bodies with ultraviolet microscopy.

Benda stain in Opisthacanthus, found that the centrioles took on a violet color, similar to that of the kinetochore (or centromere). He suggested that there might be a relationship between the centriole and the kinetochore. Bělař (4) also found that in chromosomes of living material of Chorthippus, the occurrence of idential clear areas around the centriole and the kinetochore (commisural cup (40)) was suggestive of such a relationship. Such a relationship is not evident, however, in the studies of Gelei (17) with Dendrocoelum lacteum and those of Mohr (28) with Locusta viridissima. Darlington (13), on the other hand, concluded that it was possible that the centromeres and centrosomes "are similar in their innate properties, and that the differences in their behavior are due to the difference in their position in the cell" (p. 299). However, in more recent studies of Vivipara malleatus, Pollister (30), and also Pollister and Pollister (31), provided additional information on the possible relationship between centriole and centromere. They observed that the number of extra centrioles per cell was proportional, on a one-to-one basis, to the number of acentric chromosomes. They concluded, therefore, that the extra centrioles in Vivipara were detached centromeres or "centromeric centrioles." It was noted that a centromere in the cytoplasm alongside a centriole behaved exactly like a centriole. Moreover, between centrioles and centromeres there existed a distinct similarity of size, staining reaction, and appearance in living cells. These similarities gave the Pollisters strong evidence that the centriole and centromere were similar bodies, which behaved differently, because one was in the cytoplasm

and the other a part of a chromosome. Weijer et al., (46) noted that the somatic chromosomes of N. crassa were uniformly stained and no centromere could be differentiated. It was therefore not surprising that the centrioles were Feulgen-positive, since in the case of N. crassa they are possibly evolutionary derivatives of and homologues with DNA-containing centromeres. The occurrence of DNA in the centromere is not limited to N. crassa. Lima-de-Faria (22), using the Feulgen staining technique, observed DNA in the centromeres of Tradescantia paludosa and T. virginiana.

MATERIALS AND METHODS

1. Materials

For the present study the wild-type strain of Neurospora crassa FGSC 262* and the aconidial strain FGSC 16* were used.

Vogel minimal medium (41), supplemented with trace elements and biotin, as described in "Stanford Neurospora Methods" (38), was used for the maintenance of the stock cultures.

Ryan's modification of Westergaard's minimal medium (33), but with a phosphate source, biotin, trace elements, and 2% sucrose as outlined in "Stanford Neurospora Methods" (38), was used as a crossing medium.

The Feulgen stain was prepared as follows (21, p. 199):

Basic fuchsin - 5 g.

$Na_2S_2O_5$ - 9.5 g.

0.15 N HCl - 500 ml. (room temperature)

The resulting solution was shaken for two hours. Two and a half grams of activated carbon were added and after two minutes of shaking, the suspension was filtered. The filtrate was stored in an air-tight amber bottle at 4^{o} C.

The half-oxidized Heidenhain's haematoxylin was prepared according to the method prescribed by Baker and Jordan (3).

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Observations and microphotographs were made with a Leitz Ortholux II microscope fitted with a Leitz plano objective (100X, N.A. 1.32), a Leitz apochromatic objective (90X, N.A. 1.40), Leitz periplan 10X or 12X compensating oculars, and a Leitz Aplanatic condenser (N.A. 1.40). The Leitz Xenon lamp and a low-voltage filament bulb (6 volts, 30 watts) were used interchangeably as light sources. Microphotographs were taken with either a Leitz Orthomat 35 mm automatic camera or a Leitz Aristophot bellows camera with a Leitz photo-ocular (8X). Interference filters (540 μ and 576 μ) were also used. Gevaert Scienta 50 B 65 35 mm film was employed in the Orthomat camera, and Kodak Contrast Process Ortho film (8.3 x 10.8 cm) in the Aristophot. The 35 mm film was developed with Gevaert Refinex developer and the Kodak Ortho film with Kodak D11 developer. The enlargements were printed on Kodak and Ansco paper of suitable gradations.

2. Methods

The two strains (FGSC 262 and 16) were stored separately on minimal medium agar slants at 4° C, after 3 days of initial growth at 30° C. Cultures for cytological examination were grown on filter paper "squares" (7 x 8 cm), which were partially immersed in liquid crossing medium, in sterile half-pint milk bottles stoppered with cotton plugs.

The bottles were inoculated with hyphal material of strain FGSC 16 and incubated at 23° C for about 6 - 7 days to allow protoperithecial development. Conidia of strain FGSC 262 were applied by means of

dusting to the developing protoperithecia. The dusted cultures were incubated at 23° C for 128 - 130 hours. It was found that the nuclear divisions occurred in high frequencies within this 2 hour period. At this time, sections of the filter paper, now containing growing perithecia, were cut and removed and the bottles were stored at 4° C for further use. It was found that even after 3 weeks in cold storage, the required division stages were still present in high frequencies.

The perithecia were removed one at a time from the cut sections of filter paper with a needle and placed on a slide in a drop of distilled water. Under a Leitz binocular microscope (12.5X) the developing hymenium was dissected out of the perithecium using two pairs of fine forceps. Care was taken in tearing the perithecial wall to remove the contents intact. The hymenium was then transferred by forceps into a drop of distilled water on a coverslip. When 5 - 8 hymenia had been placed in this drop of water, they were spread out into rosettes of asci under the dissecting microscope (50X). The preparation was then dried on a slide warmer at a temperature of about 60° C.

The preparations were fixed in acetic alcohol (1 part acetic acid to 3 parts of absolute alcohol) for 15 minutes, immediately hydrolyzed in 1 N HCl at 59.5° C for 8 minutes (the temperature and timing are critical) and stained for 75 minutes in Feulgen. They were then rinsed for 5 - 10 minutes in lukewarm tap water after which they were dipped (about 3 seconds) in absolute

alcohol and mounted in diluted Euparal (1 part Euparal to 1 part Euparal Essence).

Dried preparations which were stained by haematoxylin were first immersed in Helly's fluid (2, p. 25) for 2 hours, then immersed in 2% ferric ammonium sulfate (mordant) for 6 - 18 hours, and finally stained for 6 - 18 hours. They were washed by dipping in tap water between immersions in the above solutions. After staining, the preparations were differentiated in a watery solution of picric acid under a Leitz Water-Immersion objective (10X), washed in water, immersed in 70% alcohol followed by absolute alcohol, then xylol, and finally mounted on slides with diluted Canada Balsam (1 part Canada Balsam to 1 part xylol).

Although satisfactory results were obtained with the above method of culturing, fixation and staining, alternative schedules were also used. In a few instances some of the bottles were inoculated simultaneously with both strains and perithecia were removed 7 - 9 days later. In other experiments egg albumen was added to the drop on the coverslip to act as an adhesive. The use of albumen often resulted in a reduction in clarity of the final preparation.

In an attempt to hold nuclear divisions at metaphase (25, p. 377-382), perithecia were also immersed in a 0.05M - 0.1M mercaptoethanol solution for 9 - 10 hours prior to dissection. Though metaphases were not obtained, many telophases and interphases were found. In

order to prevent starvation during this treatment, perithecia were immersed in a mercaptoethanol-Vogel minimal medium solution for the 9 - 10 hour period. Both methods, however, yielded similar results.

In an attempt to obtain more rapid fixation, the drop of water on the coverslip was sometimes quick-frozen using a CO_2 freezing table. The frozen drop was then melted by adding drops of acetic alcohol while maintaining the coverslip at a low temperature with dry CO_2. Sufficient acetic alcohol was added to melt and wash away the water. The preparation was then dried on the slide warmer.

Osmium tetroxide (1.3%) in a phosphate buffer (pH 7.4) was also used (48) in an attempt to achieve rapid fixation. The use of this fixative required longer hydrolysis times (12 - 14 minutes), but both of the above fixations gave satisfactory results.

OBSERVATIONS

Identification of centrioles during ascosporogenesis

Centrioles occur throughout the nuclear divisions of ascosporogenesis of N. crassa. Feulgen and iron haematoxylin stained preparations were used to find the most suitable stages for observation of these centrioles. In the ascogenous hyphae, prior to fertilization (Figs. 1 and 2, Feulgen staining), somatic nuclear material can be observed which clearly consist of eight stainable bodies of which one is triangular in shape. At a slightly later stage (Fig. 2), a Feulgen stained preparation shows that the nuclei are thread-like, although they need not necessarily be in the same karyokinetic stage. Figure 2 shows, at the left, a filamentous nucleus belonging to Maturation cycle I (Needle phase), whereas the nucleus situated on the right side in the ascogenous hypha is in pre-division stage (Maturation cycle I) with a large centriole preparing to divide.

It became apparent from observations, that somatic configurations of different karyokinetic stages can be present in ascogenous hyphae. For instance, in young ascogenous hyphae, ring shaped nuclei may be present (Fig. 3, Feulgen staining). Since ring shaped somatic nuclei incorporate the centriole within their ring structure, microscopic differentiation of the centriole often becomes difficult.

After the invasion of two nuclei of opposite mating type into the ascogenous hypha (Figs. 4 and 5, Feulgen staining), conjugate

division takes place. Usually, the nuclei present in the ascogenous hypha appear to be in the same karyokinetic stage, indicating a certain degree of synchrony in nuclear division. Often, only a few centrioles can be observed in these ascogenous hyphae. Figure 4 shows a young ascogenous hypha in which either one pair or two individual centrioles were detected. A similar situation was observed in the hypha pictured in Figure 5. Hence, the appearance of only a few centrioles in an otherwise multinucleate ascogenous hypha, seems to indicate that nuclear synchrony is not absolute.

Throughout the examination of preparations, it was noticed that the nuclear stage does not bear any relationship to the size of the ascogenous hypha in which it is contained. For instance, the nuclear configuration in the ascogenous hypha pictured in Figure 1 is approximately in the same karyokinetic phase of development as those contained in the ascogenous hypha pictured in Figure 2. The actual hyphal structure, however, is more elaborate in Figure 1, when compared with the one pictured in Figure 2. Identical stages of ascosporogenesis are approximately identical in size within a particular rosette of asci, but may differ widely among rosettes (Fig. 4 vs Fig. 2).

The fusion nucleus is in an unsuitable nuclear configuration for the observation of centrioles. The penultimate cell is of extreme optical density when stained with either Feulgen or iron haematoxylin, so that observation of the nuclei in situ is difficult (Fig. 3). During meiosis (Division I) the dense character of the developing ascus persists and the only successful observations of

centriole development and morphology were made on squashed preparations.

Of the mitotic divisions (II, III, IV), it is especially Division IV in the developing ascospore, which is suitable for observation. Figures 6 and 7 (Feulgen staining) show interphase nuclei prior to Division IV. The centriole lies at some distance from the main nuclear configuration and there is some indication that the centriole is actually connected to the main nuclear body (Fig. 6). At the early prophase stage of Division IV (Fig. 8, iron-haematoxylin staining), the newly divided centrioles can be observed and an internal centriole spindle is present. At the time of centriole division, the nucleolus has already developed. Somewhat later (Fig. 9, iron-haematoxylin staining), the divided centrioles have separated while the chromosomes are in early prophase. Figure 10 (iron-haematoxylin staining) shows that the large triangular shaped centrioles have moved to opposite poles, with the nuclear material being organized at the equatorial plate together with two large nucleoli. Figure 11 shows an iron-haematoxylin stained preparation in which the nuclei are in late anaphase (the nuclear material is orientated in an oblique fashion; Division IV). It is evident that this particular stage is not suitable for the observation of centrioles because some of the nuclear material has already arrived at the poles and may have obscured the centrioles.

The influence of DNase on the staining of DNA-containing cellular structures

Since the main interest of this study concerns the presence of DNA (deoxyribonucleic acid) in centrioles during ascosporogenesis, it was felt that a test for DNA specificity of the Feulgen staining technique was essential. Rosettes of asci were treated with DNase (deoxyribonuclease) according to the method of Dr. I.E. Young*. Figure 12 shows the result of treating a preparation with DNase followed by Feulgen staining. It is evident that after treatment with DNase, no Feulgen positive structures are present in either asci, ascospores, or other hyphal material.

The centriole of Division I (Reduction Division)

The centriole morphology during Division I of ascosporogenesis can be readily studied in Feulgen- as well as in iron-haematoxylin-stained preparations. Figure 13 (Feulgen staining) illustrates the classic triangular shape of the centriole during early metaphase. As stated earlier, there is some indication that at least during part of its existence, the centriole is attached to the main nuclear structure. It is possible, however, that the situation as depicted in Figure 13 is due to an overlapping of a chromosome and the centriole. In Feulgen-stained squashes (Fig. 14) the centriole appears as an ovoid to spherical body. Prior to

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* Personal communication.

chromosome division, the centriole divides and the division products can be observed situated next to one another. The chromosomal configuration belonging to this centriole stage can be described as being in late prophase. A slightly later phase is depicted in Figure 15 (Feulgen staining). The centrioles have taken on an elliptical - triangular shape and appear through the microscope as vesicles with a characteristic membrane. They are deeply stained and have moved apart. The chromosomes belonging to this phase are still in late prophase. The process of centriole movement (movement towards the future spindle poles) continues (Fig. 16, Feulgen staining). The morphology of the centrioles remains approximately the same during this movement, while the chromosomes remain in late prophase. At the time of early metaphase, the centrioles become larger and take on their characteristic triangular shape (Fig. 17, Feulgen staining), while the chromosomes have started to arrange themselves along the equatorial plate. Differences in cytoplasmic density (although not Feulgen-positive) are an indication that spindle formation has started. During metaphase, the chromosomes condense at the equatorial plate and the centrioles belonging to this configuration become enlarged and deeply stained. They tend to lose their typical triangular shape and take on a more globular morphology (Fig. 18, Feulgen staining). During metaphase the centrioles are again smaller and there are some indications that a Feulgen-positive strand interconnects the main chromosomal body with the centriole (Fig. 19, Feulgen staining). At late anaphase (Fig. 20, Feulgen staining) the daughter chromosomes have moved to the spindle poles and tend to obscure the centrioles.

The centriole of Division IV (mitotic division)

The centrioles during Division IV of ascosporogenesis, i.e. during the formation of the binuclear stage of the ascospore, can be readily observed during metaphase. Figure 21 (Feulgen staining) shows deeply stained centrioles located at the spindle poles, while the chromosomes are arranged at the equatorial plate. A similar configuration is depicted in Figure 22 (Feulgen staining). Since the outline of the ascospore wall is not present in the latter photograph, it is suspected that it represents a metaphase of either mitotic division II or III. At telophase, the centrioles are globular in shape and accompany the daughter nuclei at some distance (Fig. 23, Feulgen staining). A similar situation can be observed at late telophase - early interphase (Figs. 24, 25, 26, 27, Feulgen staining). When compared with Division IV metaphase, the volume of the centrioles seems to have decreased during the course of nuclear division. Often, the centrioles are located in such a manner that they must have given rise to an oblique spindle, characteristic for Division IV of ascosporogenesis. However, it is not uncommon to find centrioles to lie to one side of the two late telophase - early interphase nuclei (Figs. 28, 29, Feulgen staining). This finding may indicate that after completion of the division, centrioles move at random around the daughter nuclei. When comparing the size of the centrioles occurring during Division IV with those of Division I, it is evident that the overall volume of the centrioles has decreased considerably during the course of ascosporogenesis. Such a decrease in centriole volume is correlated with a similar decrease in chromosomal volume.

DISCUSSION

Raper and Esser (32), in their recent treatise on the fungi, wrote: "Cytologically, the fungi have been found to differ from other organisms only in minor details, and the significance of present cytological work on fungi appears to lie in the exploitation of small differences rather than the clarification of common features" (p. 157).

The above statement might be correct with respect to the cytology of ascosporogenesis of N. crassa. However, when looking at the entire life cycle of the mold, one tends to disagree with the generalization that the cytological phenomena differ only in minor detail from those found in other organisms. The recent studies by Weijer et al., (46) show at least three different modes of karyokinesis to be operative in the vegetative cycle, all of which differ substantially from a classical type of mitosis. In addition, the prediction was made that the centrioles, commonly found in fungi, contain DNA*.

Centrioles, together with chloroplasts and mitochondria, belong to the group of extra-nuclear bodies. As stated earlier, DNA has already been detected in chloroplasts as well as in mitochondria (see p. 2, 4). Therefore, the original finding by Weijer et al., (46) that the centrioles of the somatic nuclei have a positive Feulgen reaction, is in line with the universal notion that all cellular bodies showing independent self-duplication, contain the genetic information (DNA) necessary to do so. From the studies of

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* One of the earliest accounts concerning centrioles in Neurospora was published by Dodge (15) in his paper: "Nuclear phenomena associated with heterothallism and homothallism in the Ascomycete Neurospora." Figure 30 is a photographic reproduction of one of the plates presented in this paper.

Bucher and Mazia (6), it became evident that the centriole and the nucleus are independent entities, and that the formation of chromosomal DNA does not seem to depend on the antecedent duplication of the mitotic centers. A similar conclusion was reached by Weijer and Koopmans (44) concerning the centriole of the somatic nucleus of N. crassa. Their results clearly showed that chromosomal DNA replication precedes the visual centriole duplication and the rotation of its divisionary products. It seems plausible, however, that the replication of centriole DNA coincides with the replication of chromosomal DNA. It other words, the cellular enzyme system which triggers the replication of chromosomal DNA likewise affects the DNA of the centriole.

Due to the thread-like morphology of the karyokinetic structures during the Juvenile-, Maturation I-, and Maturation II cycles, microscopic differentiation of the centriole is difficult. Its shape in the vegetative cycle closely resembles that of somatic chromosomes and differs only with respect to its stainability. In hyphal preparations, the centriole appears as a deeply-stained, Feulgen-positive body.

Although geneticists have presented evidence that there are only seven linkage groups in Neurospora which account for the seven chromosomes, the possibility that a genetically undetected eighth linkage group could exist, cannot be ruled out. The existence of such an eighth linkage group would then, in turn, explain the appearance of eight stainable bodies within a nuclear

configuration. This explanation, for instance, was favored by Somers et al.,(37). The difficulty of microscopic differentiation, however, does not arise when examining the divisions belonging to ascosporogenesis. Especially during Division I (the Reduction Division), the recognition of the centrioles is facilitated by the contrasting morphology of the chromosomes preparing for division. Within these plates (Figs. 14 - 17), the centrioles appear as deeply stained, Feulgen positive bodies, surrounded by typical prophase chromosomes. Since the intensity of the Feulgen stain is an indication of the quantity of DNA present, it can be concluded that a surprisingly large amount of DNA is present in the centriole.

The subsequent mitotic divisions (Divisions II, III and IV) are less suitable for centriole observation. Due to the fact that the chromosomes of these mitotic divisions are very small, and of the same magnitude as the actual mitotic centrioles, a clear-cut identification of the extra-nuclear bodies becomes difficult. For instance, the photographs, as presented in Figures 21 - 29, can be interpreted as showing telophase nuclear configurations in which two lagging chromosomes or chromosomal fragments are apparent. Such an explanation, however, would be inconsistent with some of the other pictorial evidence presented in this thesis. In Figures 21 - 29 the two lagging elements of the telophase configuration are situated between the daughter nuclei. The metaphase configurations in Figures 19 and 20, on the other hand, show the same elements at the poles, indicating (in accordance with the above explanation)

that the main chromosomal mass is now lagging behind. The inconsistency of this explanation, together with the observations made during the Reduction Division, invalidates the above postulate.

In some of the preparations studied (Figs. 6, 13 and 19), there is an indication (although not conclusive) of a connection (probably protein in nature) between the centriole and the main part of the nucleus. During prophase I especially, this connection may be very short, resulting in an almost immediate attachment of the centriole to one of the chromosome arms (Fig. 13). Moreover, the common tendency of centrioles to be located close to the daughter nuclei at late telophase and early interphase of Division IV, strengthens this view.

Additional evidence for a physical connection between the centriole and nucleus was gathered by Weijer and McDonald (47) in preparations of late telophase and early-mid interphase of Division IV. Towards the end of Division IV, the daughter nuclei assume the atypical ring shaped appearance which subsequently gives rise to the "broken ring" or "Horseshoe" phase. This phase consists clearly of eight Feulgen positive bodies (seven chromosomes and the centriole) arranged in a linear sequence along a thread-like structure. Therefore, Division IV is only partly classical in its mode of division and terminates in nuclear structures which are characteristic for the early phases of the atypical karyokinesis of the Juvenile cycle.

Singleton (36), in his classical paper dealing with chromosome morphology and the chromosome cycle in the ascus of N. crassa, states

that: "Between metaphase I and metaphase III, the centriole undergoes a relatively enormous increase in size and volume" (p. 142). The results presented in this paper, however, do not confirm entirely such an observation. It was found that during the Reduction Division the centrioles reach their maximum size. During Divisions II and III they are relatively small, whereas during Division IV the centrioles do increase in size. From a genetic, as well as from a cytological point of view, one would expect the maximum size of the centrioles to coincide with nuclear fusion. During fertilization, fusion of the centrioles belonging to nuclei of opposite mating types should occur, and hence the amount of genetic material (DNA) in the fertilization centriole should be twice the amount present in the centriole of the telophase nuclei of the mitotic divisions. Another increase in size of centrioles should be reached during centriole DNA replication prior to mitotic divisions. However, the "2n" phase of the centriole in Divisions II, III and IV is probably much shorter than the one occurring prior to the Reduction Division.

The finding that the centrioles of N. crassa contain DNA as their genetic determinant is of evolutionary importance: it provides additional evidence for the postulate of the Pollisters (30, 31) that the centriole, on structural as well as theoretical grounds, is homologous with the centromere. Since it has been known (22) that the centromere granule is Feulgen positive (confirmed in N. crassa by Weijer et al., (46)), these grounds can now be extended to include chemical homology.

BIBLIOGRAPHY

1. Amano, S. 1954. Structure and function of the central body and the nucleolus. Extension-fiber theory of the mitotic mechanism. Acta Schol. Med. Univ. Kioto 32:5-18.

2. B.D.H. Standard Stains, The British Drug Houses (Canada) Ltd. Toronto, Canada. 53 pp.

3. Baker, J.R., and Jordan, B.M. 1953. Miscellaneous contributions to microtechnique. Quart. J. Microbiol. Sci. 94:237-242.

4. Belar, K. 1929. Beiträge zur Kausalanalyse der Mitose. II. Untersuchungen an den Spermatocyten von Chorthippus (Stenobothrus) lineatus. Panz. Arch. Entwn. 118:359-484.

5. Berlin, J.D., and Bowan, C.C. 1964. Centrioles in the fungus Albugo candida. Am. J. Botany 51:650-652.

6. Bucher, N.L.R., and Mazia, D. 1960. Deoxyribonucleic acid synthesis in relation to duplication of centers in dividing eggs of the sea urchin, Strongylocentrotus purpuratus. J. Biophys. Biochem. Cytol. 7:651-655.

7. Chun, E.H.L., Vaughan, M.H., and Rich, A. 1963. The isolation and characterization of DNA associated with chloroplast preparations. J. Mol. Biol. 7:130-141.

8. Cleveland, L.R. 1938. Longitudinal and transverse division in two closely related flagellates. Biol. Bull. 74: 1-24.

9. Cleveland, L.R. 1938. Origin and development of the achromatic figure. Biol. Bull. 74:41-55.

10. Cleveland, L.R. 1949. The whole life cycle of chromosomes and their coiling systems. Trans. Am. Phil. Soc. 39:1-100.

11. Cleveland, L.R. 1953. Studies on chromosomes and nuclear division. Trans. Am. Phil. Soc. 43:809-869.

12. Cooper, W.D., and Loring, H.S. 1957. The ribonucleic acid composition and phosphorus distribution of chloroplasts from normal and diseased Turkish tobacco plants. J. Biol. Chem. 228:813-822.

13. Darlington, C.D. 1937. The external mechanics of the chromosomes. Proc. Roy. Soc. (London) B 121:264-319.

14. Darlington, C.D. 1937. Recent Advances in Cytology. 2nd ed., J. and A. Churchill Ltd., London. 671 pp.

15. Dodge, B.O. 1927. Nuclear phenomena associated with heterothallism and homothallism in the Ascomycete Neurospora. J. Agr. Res. 35:289-305.

16. Dodge, B.O., Singleton, J.R., and Rolnick, A. 1950. Studies on lethal E gene in Neurospora tetrasperma, including chromosome counts also in races of N. sitophila. Proc. Am. Phil. Soc. 94:38-52.

17. Gelei, J. 1921. Weitere Studien uber die Oogenese des Dendrocoelum lacteum. II. Die langskonjugation der Chromosomen. Arch. Zellforsch. 16:88-170.

18. Harper, R.A. 1897. Kernteilung u. freie Zellbildung im Ascus. J.B. f. wiss. Bot. XXX:249-284.

19. Harper, R.A. 1905. Sexual reproduction and the organization of the nucleus in certain mildews. Carnegie Institution of Washington, Publication No. 37, 105 pp.

20. Hoerr, N.L., and Osol, A. (Edits.) 1956. Blakiston's New Gould Medical Dictionary. 2nd ed. McGraw-Hill, New York.

21. Jensen, W.A. 1962. Botanical Histochemistry. W. H. Freeman and Company, San Francisco.

22. Lima-de-Faria, A. 1958. Recent advances in the study of the kinetochore. Intern. Rev. Cytol. 7:123-157.

23. Lindegren, C.C., Bang, Y.N., and Osumi, M. 1965. The central body of the Ascomycetes. Can. J. Genet. Cytol. 7:37-39.

24. Lu, B.C. 1964. Chromosome cycles of the Basidiomycete Cyathus stercoreus (Schw.) De. Toni. Chromosoma (Berl.) 15:170-184.

25. Mazia, D. 1959. The role of thiol groups in the structure and function of the mitotic apparatus. In Sulfur in proteins. Academic Press, New York. 377-382.

26. Mazia, D. 1961. Mitosis and the physiology of cell division. In J. Brachet and A.E. Mirsky, The cell. Academic Press, New York. 3:77-412.

27. McClintock, B. 1945. Neurospora. I. Preliminary observations of the chromosomes of Neurospora crassa. Am. J. Botany 32: 671-678.

28. Mohr, O.L. 1916. Studien uber die Chromatinreifung der mannlichen Geschlechtszellen bei Locusta viridissima. Arch. Biol. 29:579-750.

29. Olive, L.S. 1953. The structure and behavior of fungus nuclei. Botan. Rev. 19:439-586.

30. Pollister, A.W. 1939. Centrioles and chromosomes in the atypical spermatogenesis of Vivipara. Proc. Nat. Acad. Sci. U.S. 25:189-195.

31. Pollister, A.W., and Pollister, P.F. 1943. The relation between centriole and centromere in atypical spermatogenesis of viviparid snails. Ann. N.Y. Acad. Sci. 45:1-48.

32. Raper, J.R., and Esser, K. 1964. The fungi. In J. Brachet and A.E. Mirsky, The cell. Academic Press, New York 6:139-244.

33. Ryan, F.J. 1950. Selected methods of Neurospora genetics. In Methods in medical research 3:51-75. The Year Book Publ. Inc., Chicago.

34. Schatz, G., Haslbrunner, E., and Tuppy, H. 1964. Deoxyribonucleic acid associated with yeast mitochondria. Biochem. Biophys. Res. Commun. 15:127-132.

35. Schrader, F. 1936. The kinetochore or spindle fiber locus in Amphiuma tridactylum. Biol. Bull. 70:484-498.

36. Singleton, J.R. 1953. Chromosome morphology and the chromosome cycle in the ascus of Neurospora crassa. Am. J. Botany 40: 124-144.

37. Somers, C.E., Wagner, R.P., and Hsu, T.C. 1960. Mitosis in vegetative nuclei of Neurospora crassa. Genetics 45:801-810.

38. Stanford Neurospora methods. In Neurospora newsletter 4:21-25.(1963).

39. Stich, H. 1954. Stoffe und Strömungen in der Spindel von Cyclops strenuus. Ein Beitrag zur Mechanik der Mitose. Chromosoma 6:199-236.

40. Swanson, C.P. 1963. Cytology and Cytogenetics. 5th ed. Prentice-Hall, Englewood Cliffs, New Jersey.

41. Vogel, H.J. 1956. A convenient growth medium for Neurospora. Microbiol. Genet. Bull. 13:42-43.

42. Ward, E.W.B., and Ciurysek, K.W. 1962. Somatic mitosis in Neurospora. Am. J. Botany 49:393-399.

43. Weijer, D.L. 1964. Karyokinesis of somatic nuclei of Neurospora crassa. I. The correlation between conidial radiosensitivity and their karyokinetic stage. Can. J. Genet. Cytol. 6:383-392.

44. Weijer, J., and Koopmans, A. 1964. Karyokinesis of somatic nuclei of Neurospora crassa. II. DNA replication in synchronously dividing conidial nuclei. Can. J. Genet. Cytol. 6:426-430.

45. Weijer, J., Koopmans, A., and Weijer, D.L. 1963. Karyokinesis in vivo of the migrating somatic nucleus of Neurospora and Gelasinospora species. Trans. N.Y. Acad. Sci. 24: 846-854.

46. Weijer, J., Koopmans, A., and Weijer, D.L. 1965. Karyokinesis of somatic nuclei of Neurospora crassa. III. The juvenile and maturation cycles (Feulgen and crystal violet staining). Can. J. Genet. Cytol. 7:140-163.

47. Weijer, J., and McDonald, B.R. 1965. Karyokinesis of somatic nuclei of Neurospora crassa. IV. The occurrence of the juvenile ring phase during division IV of ascosporogenesis. Can. J. Genet. Cytol. In press.

48. Wood, R.L., and Luft, J.H. 1965. The influence of buffer systems on fixation with osmium tetroxide. J. of Ultrastruc. Res. 12:22-45.

Fig. 1. Young ascogenous hypha of N. crassa prior to fertilization. The hypha contains eight stainable bodies (7 chromosomes and 1 triangularly shaped centriole). Feulgen staining. 2200X.

c = centriole

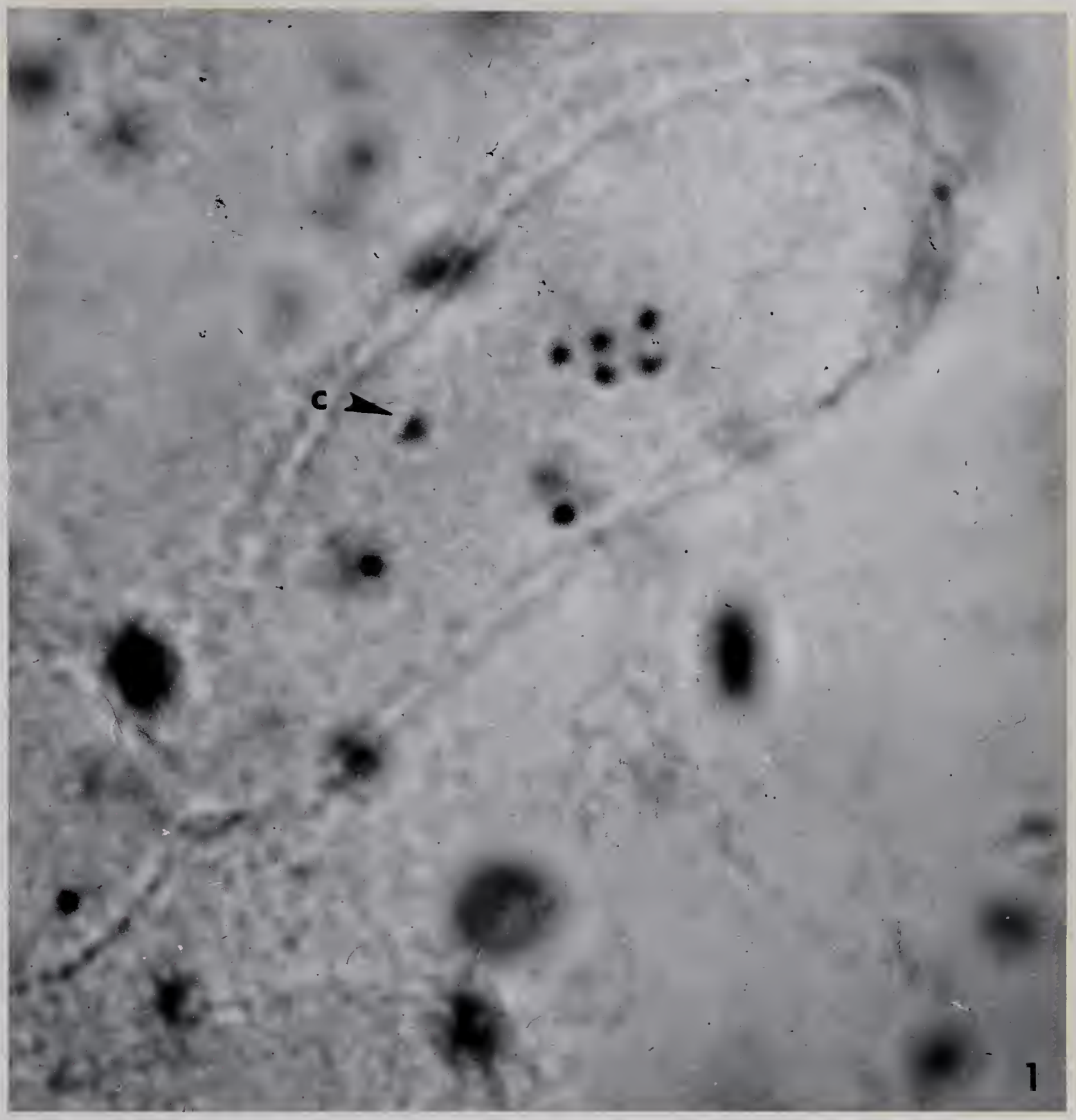
C
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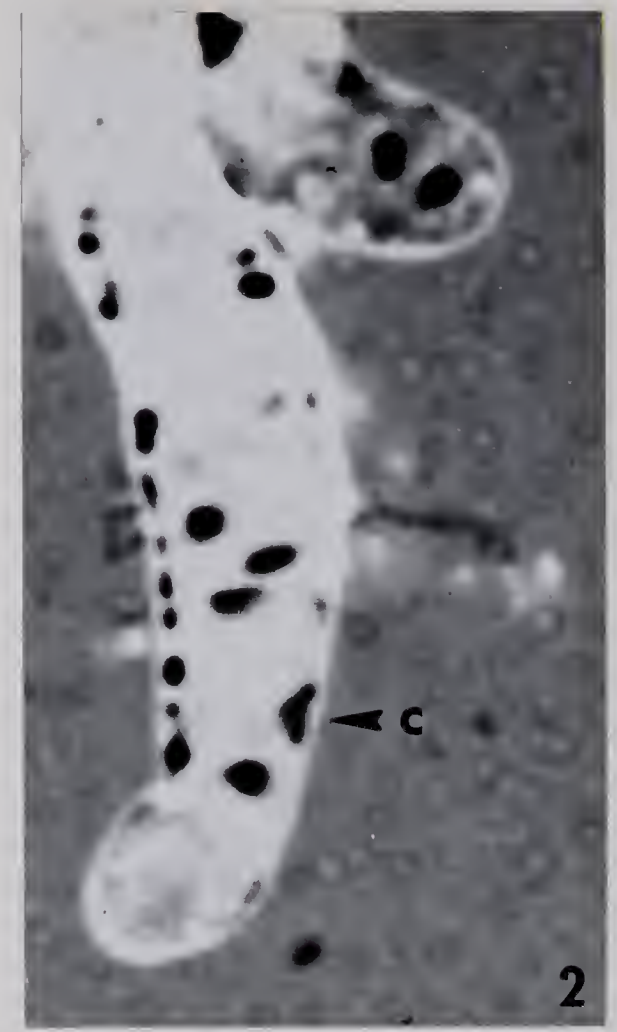
Fig. 2. Ascogenous hypha of N. crassa prior to fertilization. In the hypha, at the left, is a filamentous nucleus belonging to Maturation cycle I (Needle phase) and at the right is a nucleus in pre-division stage (Maturation cycle I) with a large centriole preparing to divide. Feulgen staining. 1600X.

Fig. 3. Young ascogenous hyphae of N. crassa prior to fertilization. Ring shaped nuclei are present in the hyphae. Feulgen staining. 1600X.

Fig. 4. Ascogenous hypha of N. crassa after conjugate division has taken place. Either one pair or two individual centrioles are detectable. Feulgen staining. 1600X.

c = centriole



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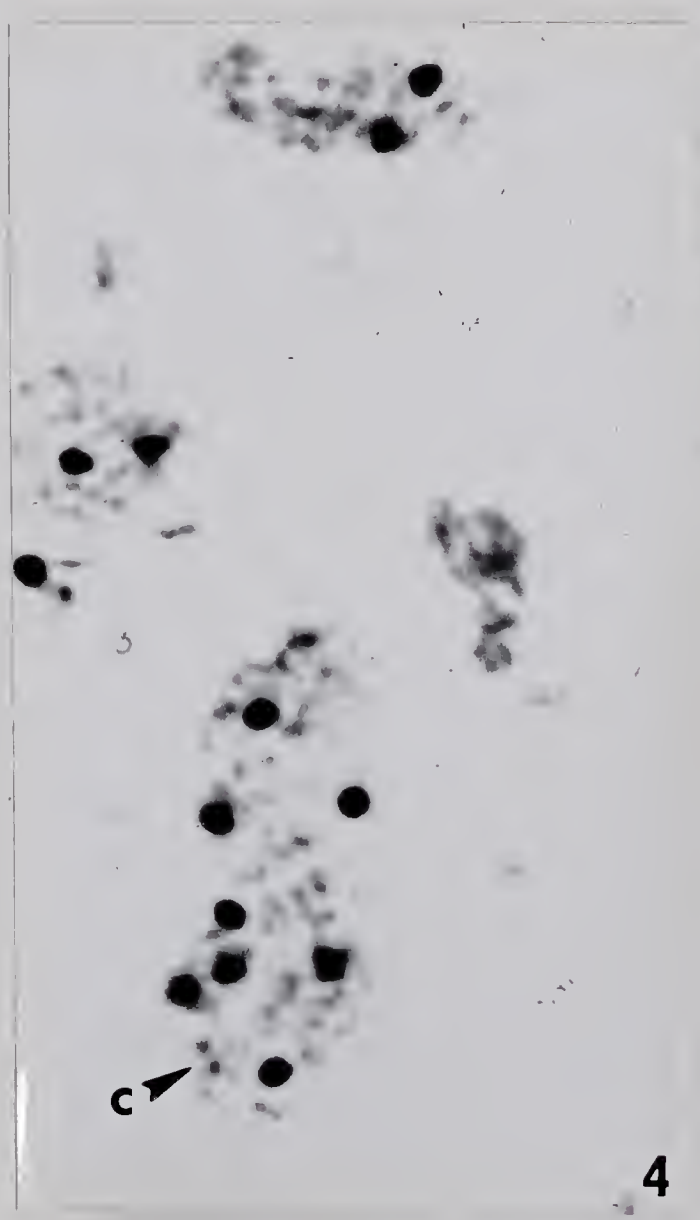

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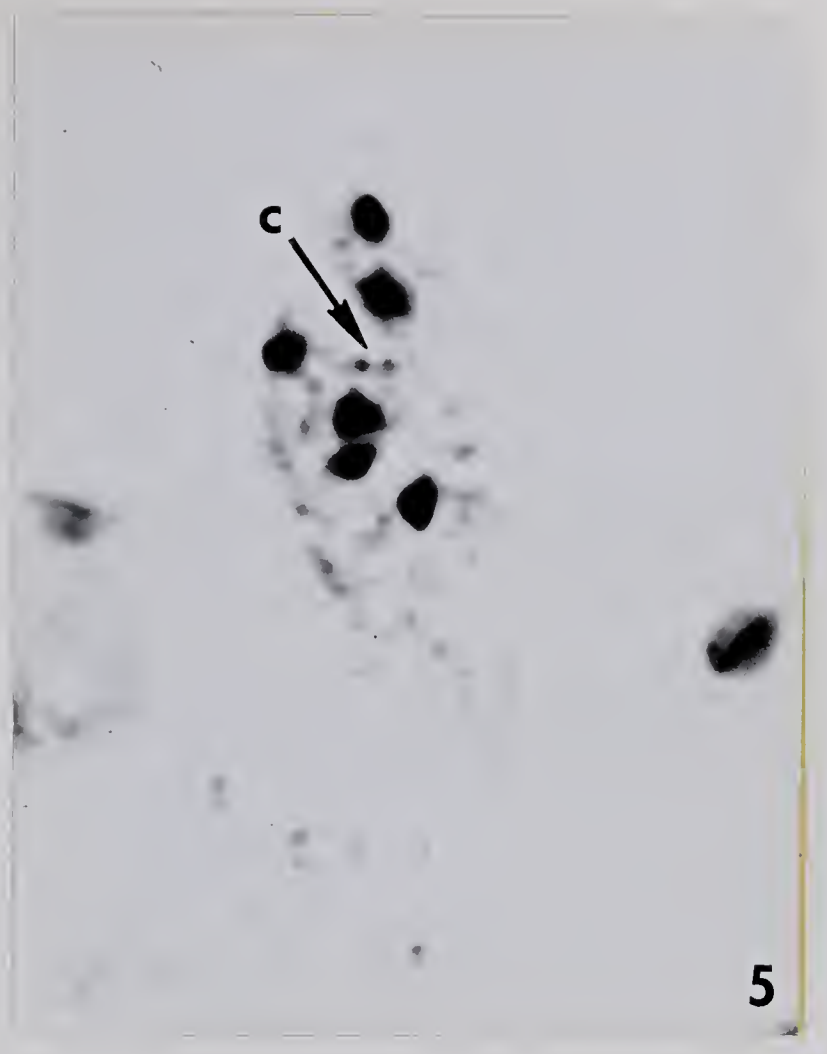
Fig. 5. Ascogenous hypha of N. crassa after conjugate division has taken place. Either one pair or two individual centrioles are detectable. Feulgen staining. 1600X.

Fig. 6. Developing ascospore of N. crassa containing an interphase nucleus prior to Division IV. Feulgen staining. 1600X.

c = centriole; n = nucleus



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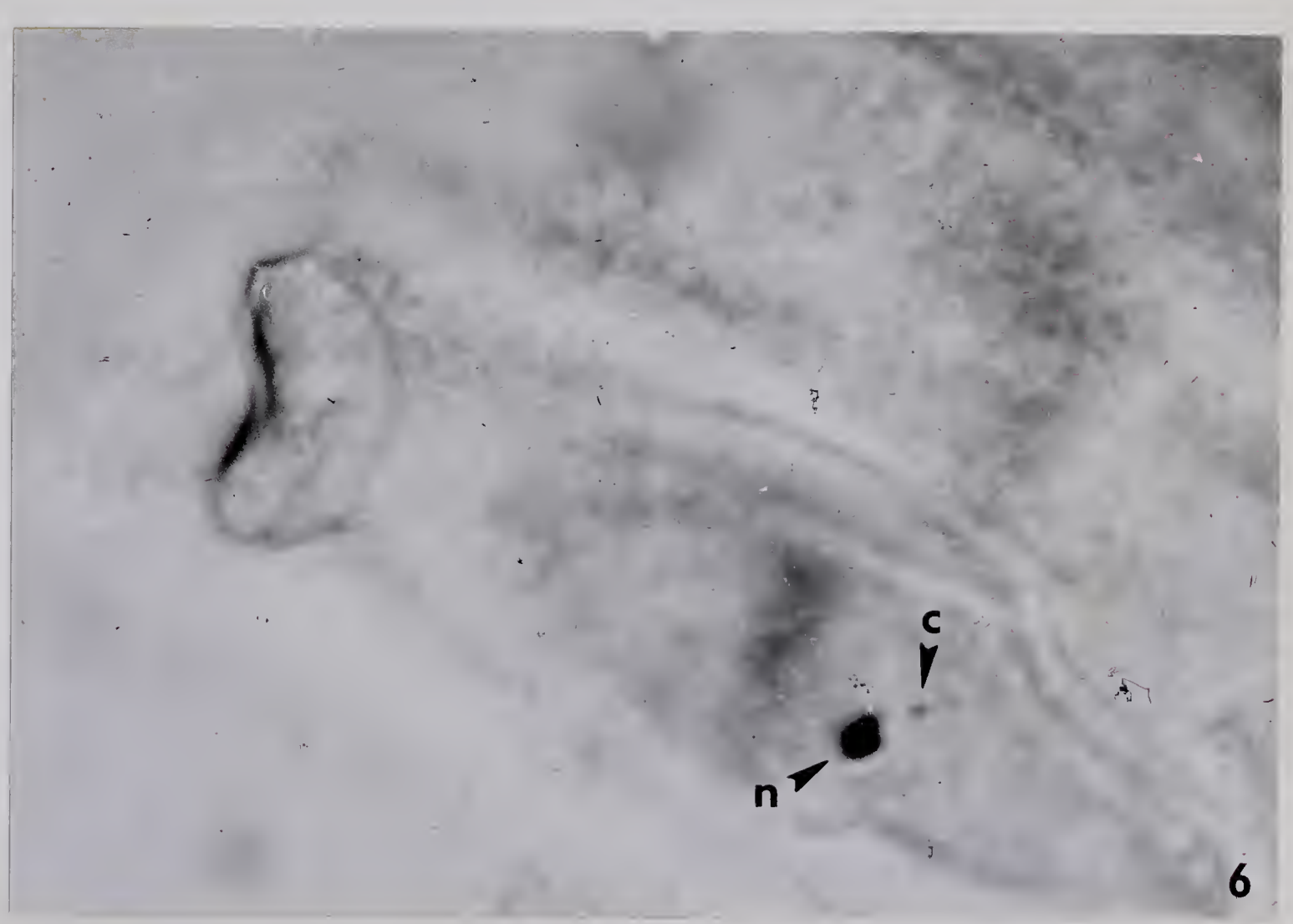

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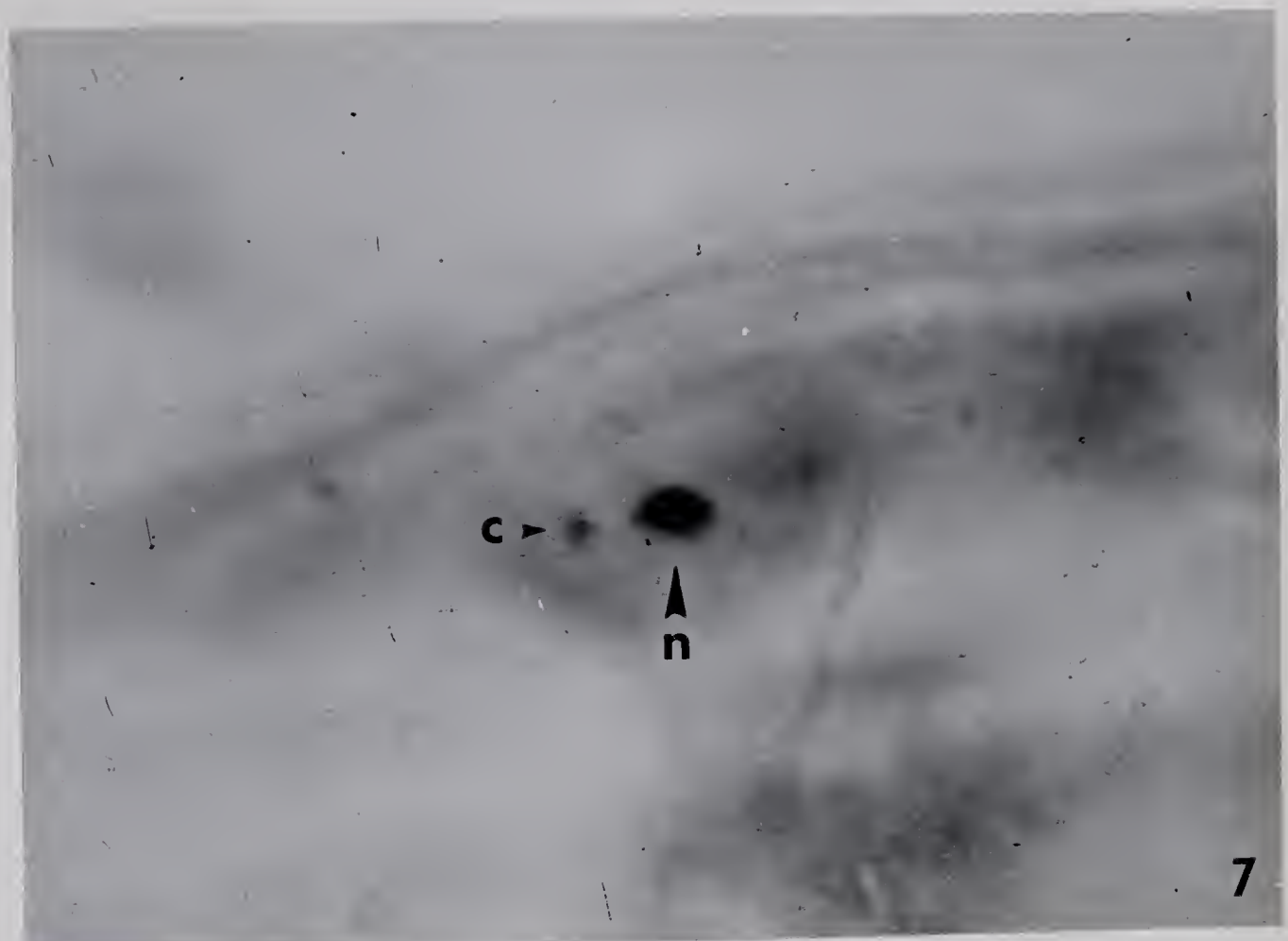
Fig. 7. Developing ascospore of N. crassa containing an interphase nucleus prior to Division IV. Feulgen staining. 1600X.

Fig. 8. Developing ascospore of N. crassa containing a nuclear configuration at the early prophase stage of Division IV. The nucleolus has already developed and newly divided centrioles can be observed with an internal centriole spindle. Iron haematoxylin staining. 2200X.

c = centriole; n = nucleus; no = nucleolus



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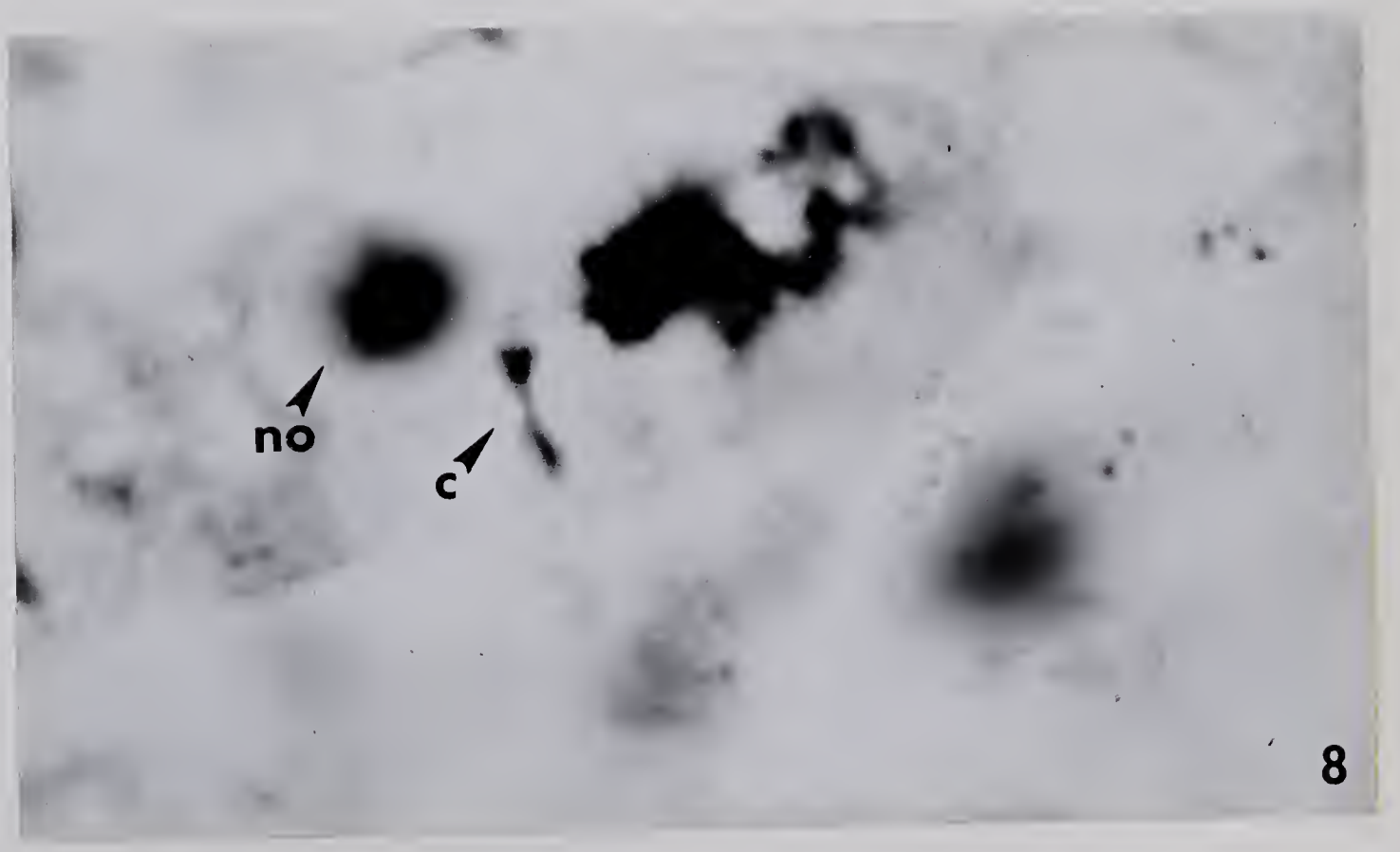

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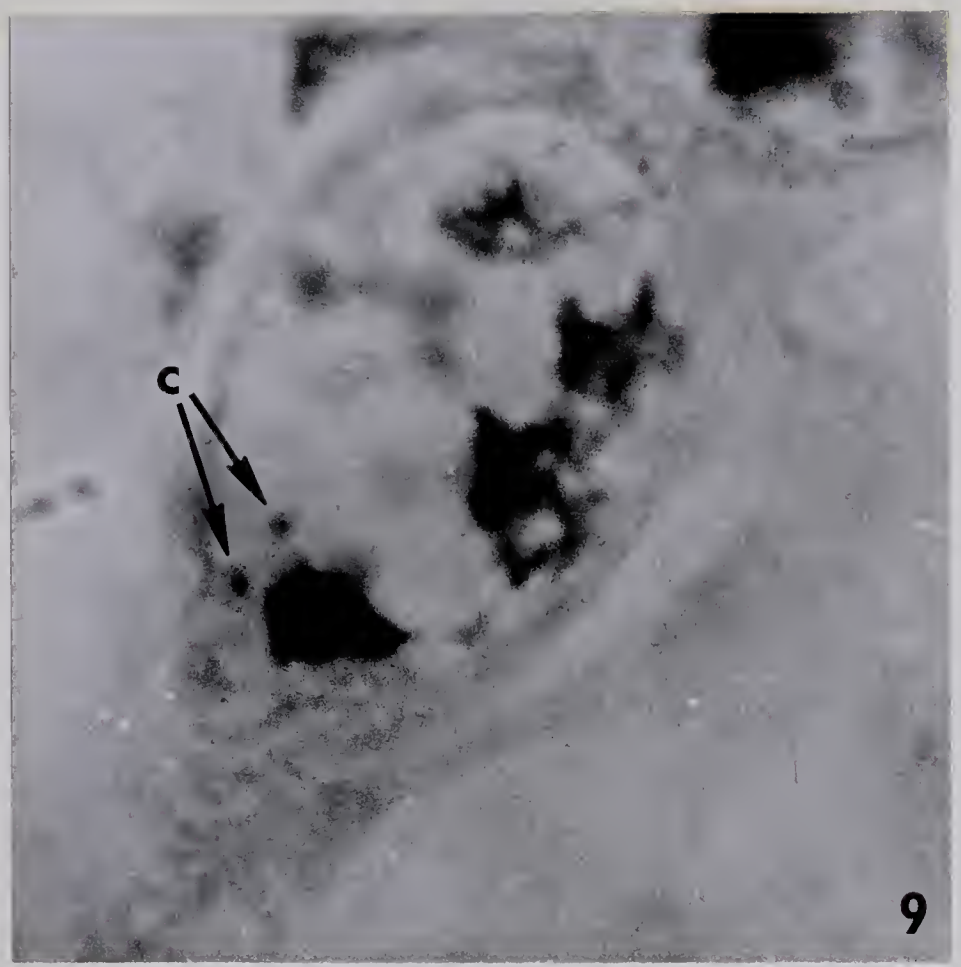
Fig. 9. Developing ascospore of N. crassa containing a nuclear configuration at the early prophase stage of Division IV. The nucleolus is present and the divided centrioles have separated. Iron haematoxylin staining. 1600X.

Fig. 10. Developing ascospore of N. crassa containing a nuclear configuration at the metaphase stage of Division IV, together with two large nucleoli. Large triangular shaped centrioles have moved to opposite poles. Iron haematoxylin staining. 2200X.

Fig. 11. Developing ascospores of N. crassa containing nuclear configurations at the late anaphase stage of Division IV. Iron haematoxylin staining. 1600X.

c = centriole; no = nucleolus



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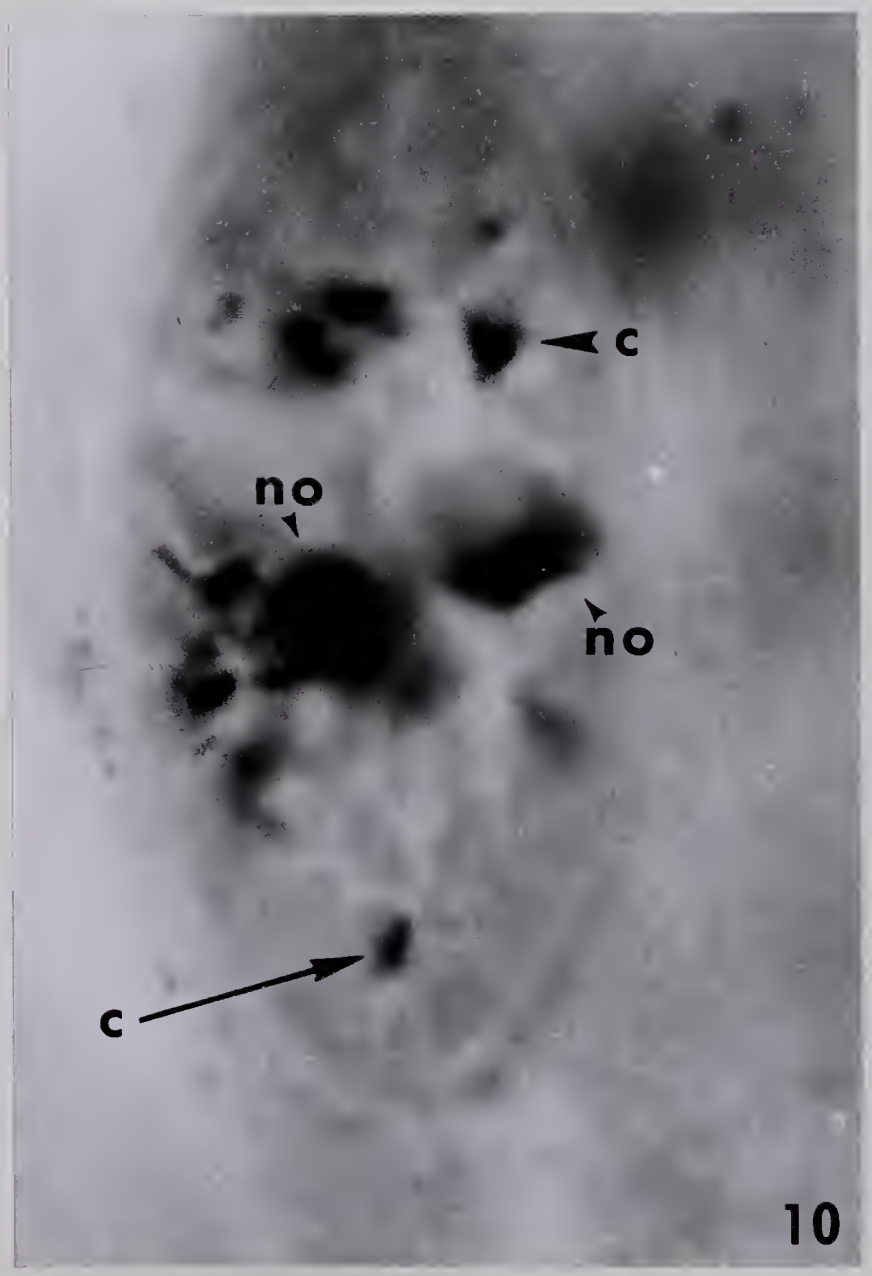

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Fig. 12. Ascospore, asci and other hyphal material of N. crassa after treatment with DNase followed by Feulgen staining. 2200X.

Fig. 13. Nuclear configuration of N. crassa during early metaphase of Division I of ascosporogenesis. The triangularly shaped centriole is either attached to the main nuclear structure or is overlapping a chromosome. Feulgen staining. 2200X.

c = centriole

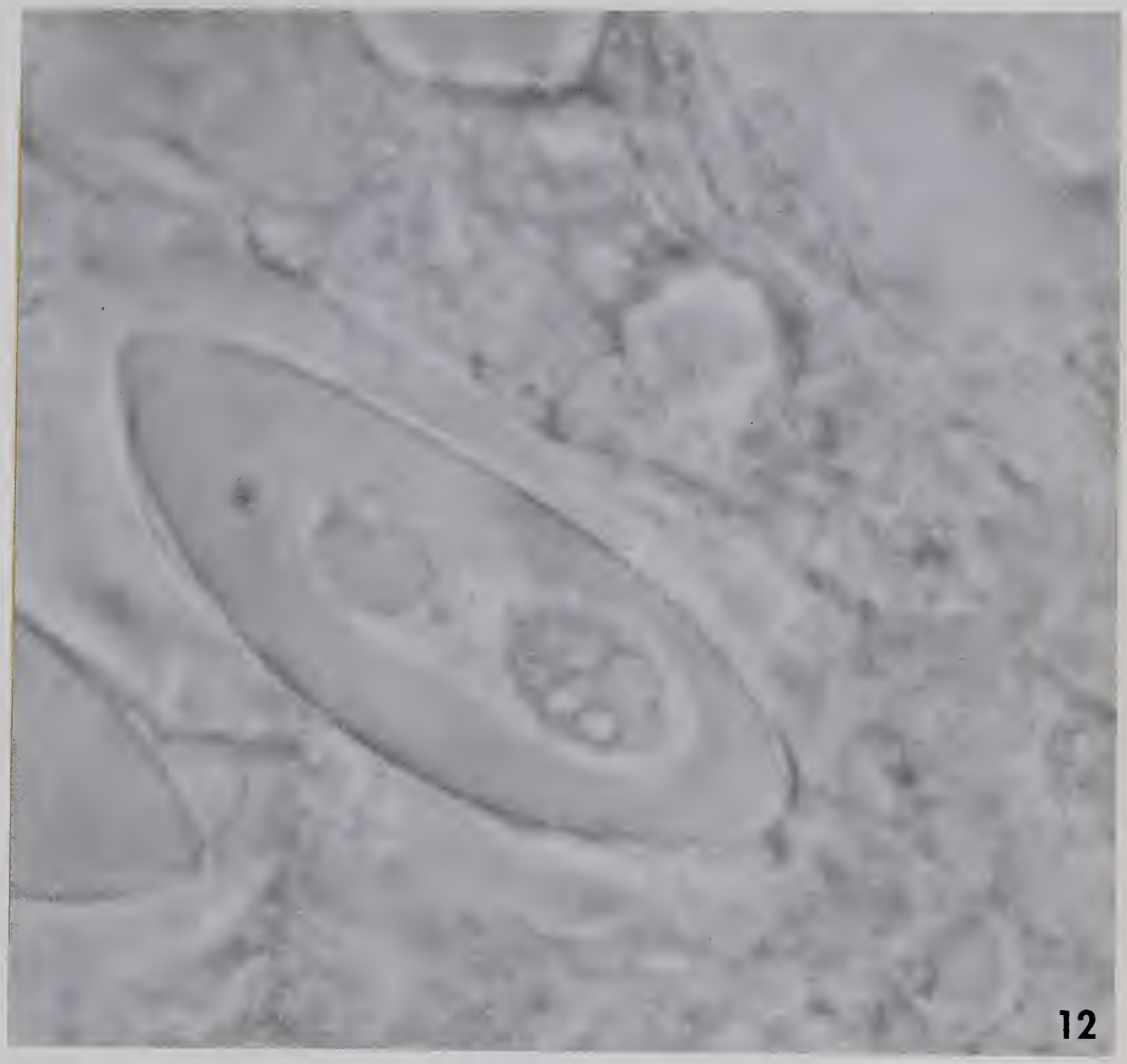

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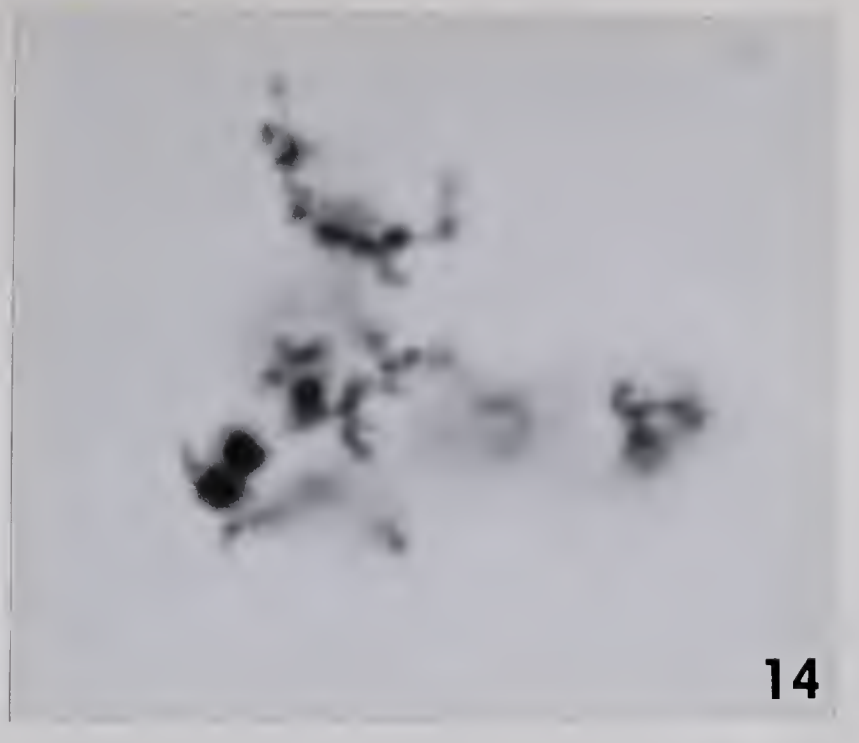
Fig. 14. Nuclear configuration of N. crassa during late prophase of Division I of ascosporogenesis. The ovoid to spherical centriole has divided and the division products can be observed situated next to one another. Feulgen staining. 1600X.

Fig. 15. Nuclear configuration of N. crassa during late prophase of Division I of ascosporogenesis. The centrioles have taken on an elliptical - triangular shape and have moved apart. Feulgen staining. 1600X.

Fig. 16. Nuclear configuration of N. crassa during late prophase of Division I of ascosporogenesis. The centrioles have moved further towards the future spindle poles. Feulgen staining. 1600X.



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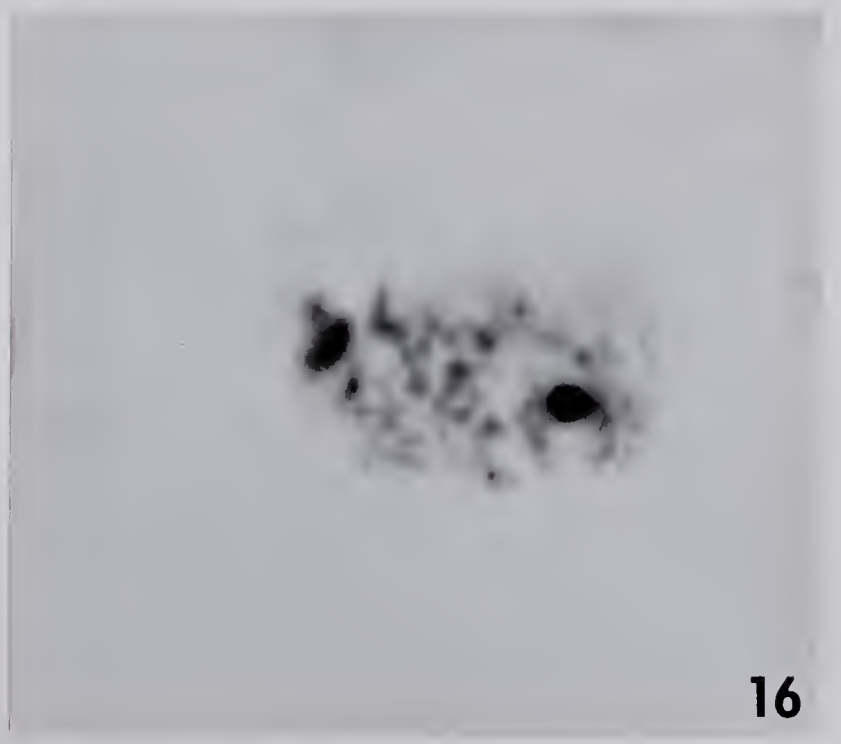

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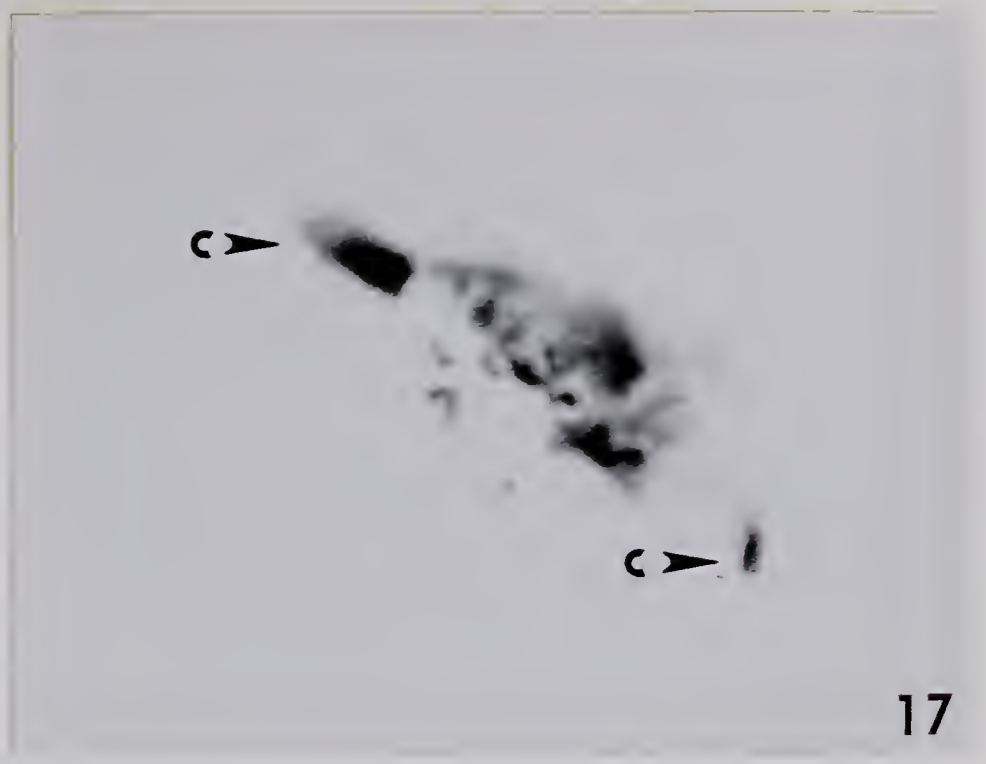
Fig. 17. Nuclear configuration of N. crassa during early metaphase of Division I of ascosporogenesis. The centrioles have become larger and have taken on their characteristic triangular shape, while the chromosomes have started to arrange themselves along the equatorial plate. Spindle formation has started. Feulgen staining. 1600X.

Fig. 18. Nuclear configuration of N. crassa during metaphase of Division I of ascosporogenesis. The chromosomes have condensed at the equatorial plate and large spherical centrioles are at the poles. Feulgen staining. 1600X.

Fig. 19. Nuclear configuration of N. crassa during metaphase of Division I of ascosporogenesis. One centriole is visible. A Feulgen positive strand possibly interconnects the main chromosomal body with the centriole. Feulgen staining. 1600X.

c = centriole; n = nucleus



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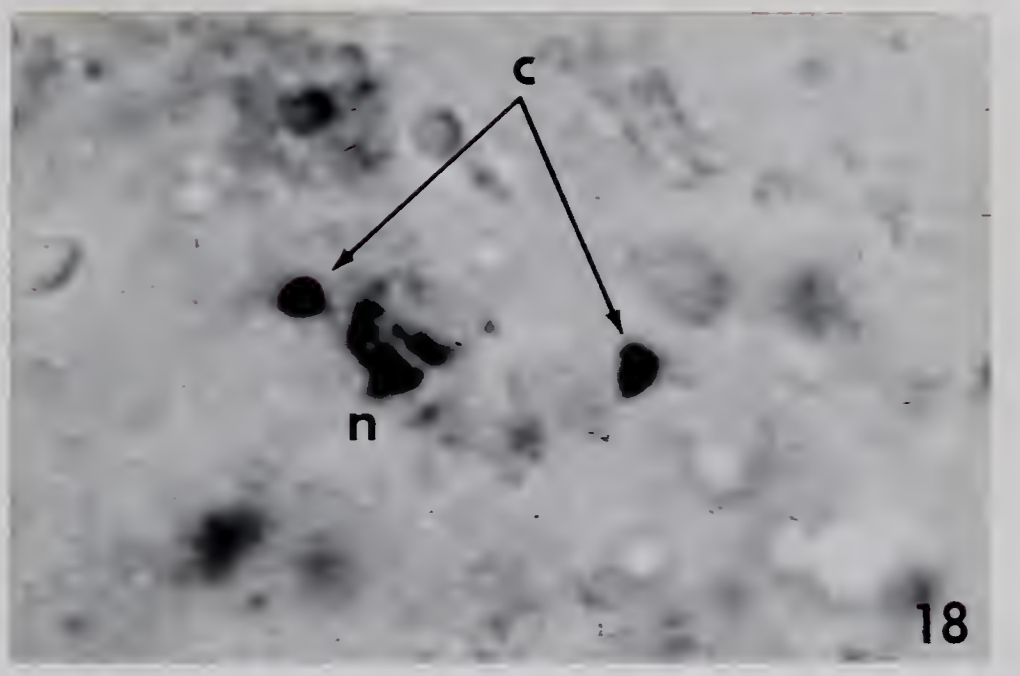

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Fig. 20. Nuclear configuration of N. crassa during late anaphase of Division I of ascosporogenesis. Feulgen staining. 1600X.

Fig. 21. Metaphase of Division IV of ascosporogenesis in N. crassa. Centrioles are located at the spindle poles, while the chromosomes are arranged at the equatorial plate. Feulgen staining. 2200X.

Fig. 22. Metaphase of either Division II or III of ascosporogenesis in N. crassa. Centrioles are located at the spindle poles, while the chromosomes are arranged at the equatorial plate. Feulgen staining. 1600X.

c = centriole

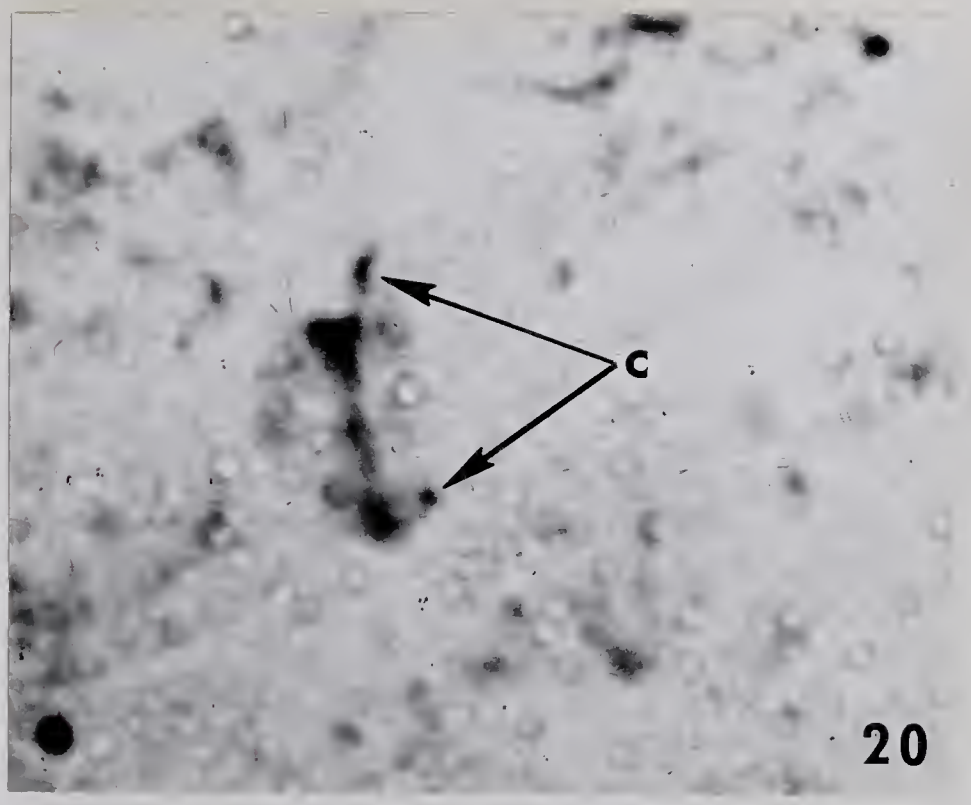

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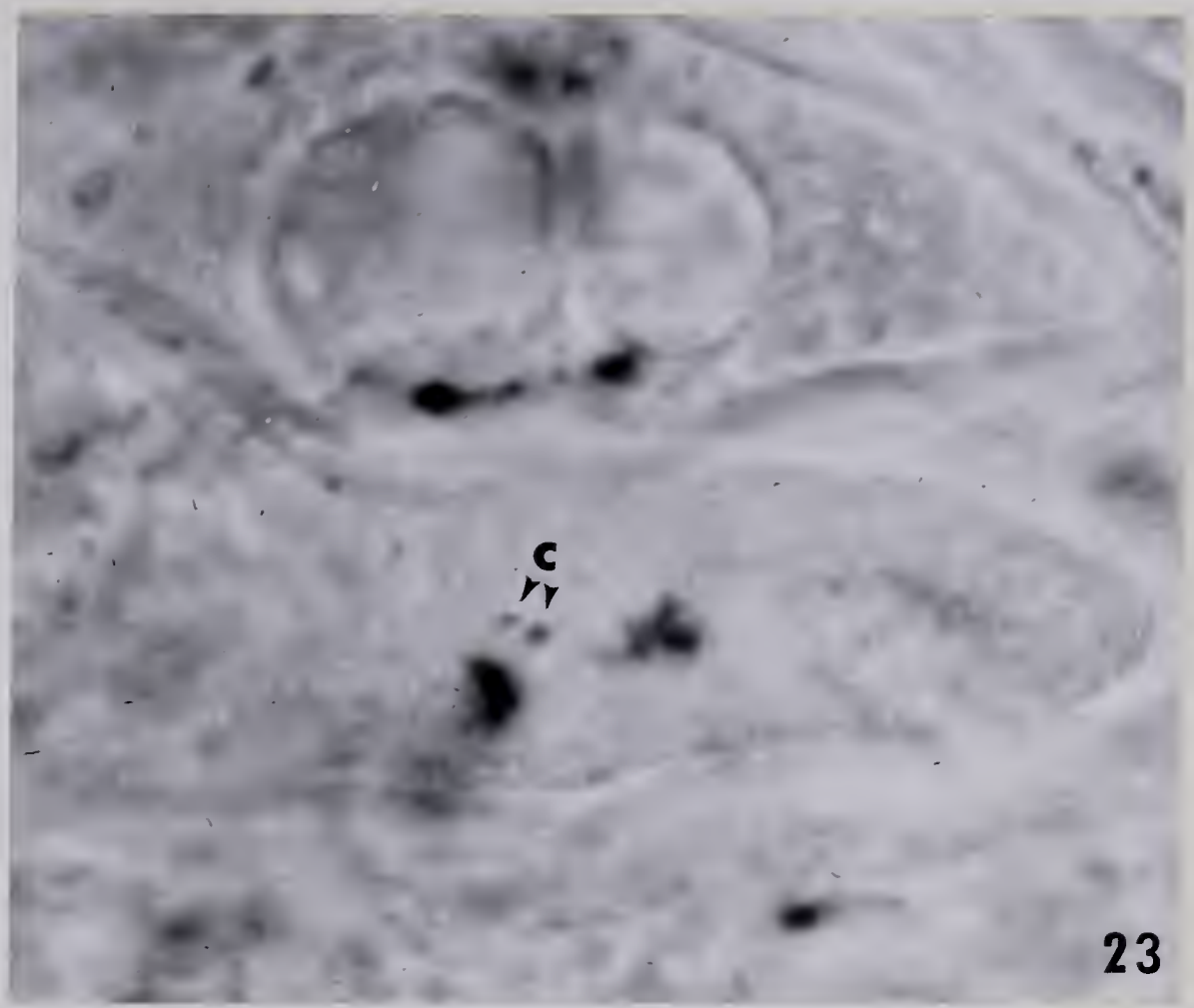
Fig. 23. Telophase of Division IV of ascosporogenesis. Two globular centrioles are visible. Feulgen staining. 2200X.

Fig. 24. Late telophase - early interphase of Division IV of ascosporogenesis. Two globular centrioles are visible. Feulgen staining. 2200X.

c = centriole



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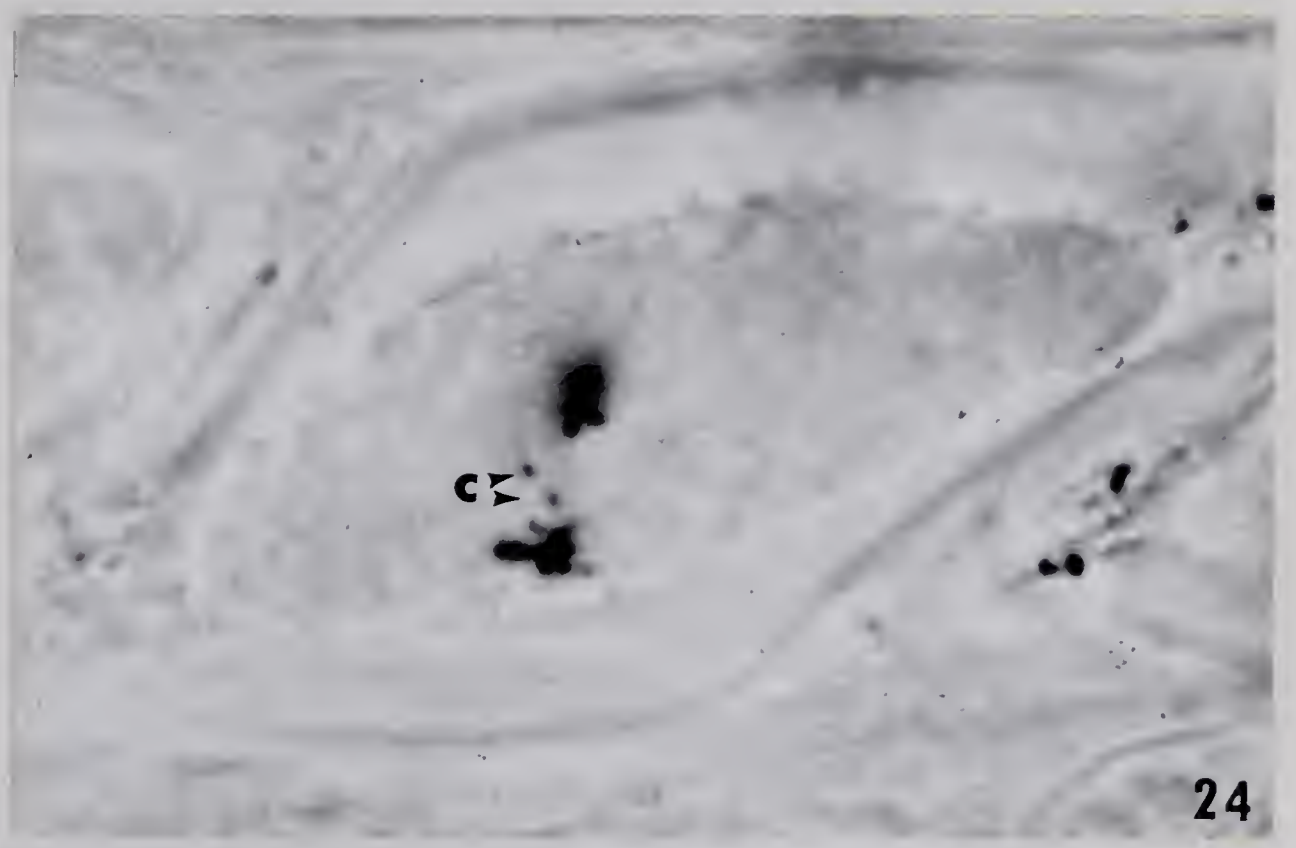

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Fig. 25. Late telophase - early interphase of Division IV of ascosporogenesis. One globular centriole is visible. Feulgen staining. 2200X.

Fig. 26. Late telophase - early interphase of Division IV of ascosporogenesis. One globular centriole is visible. Feulgen staining. 2200X.

c = centriole

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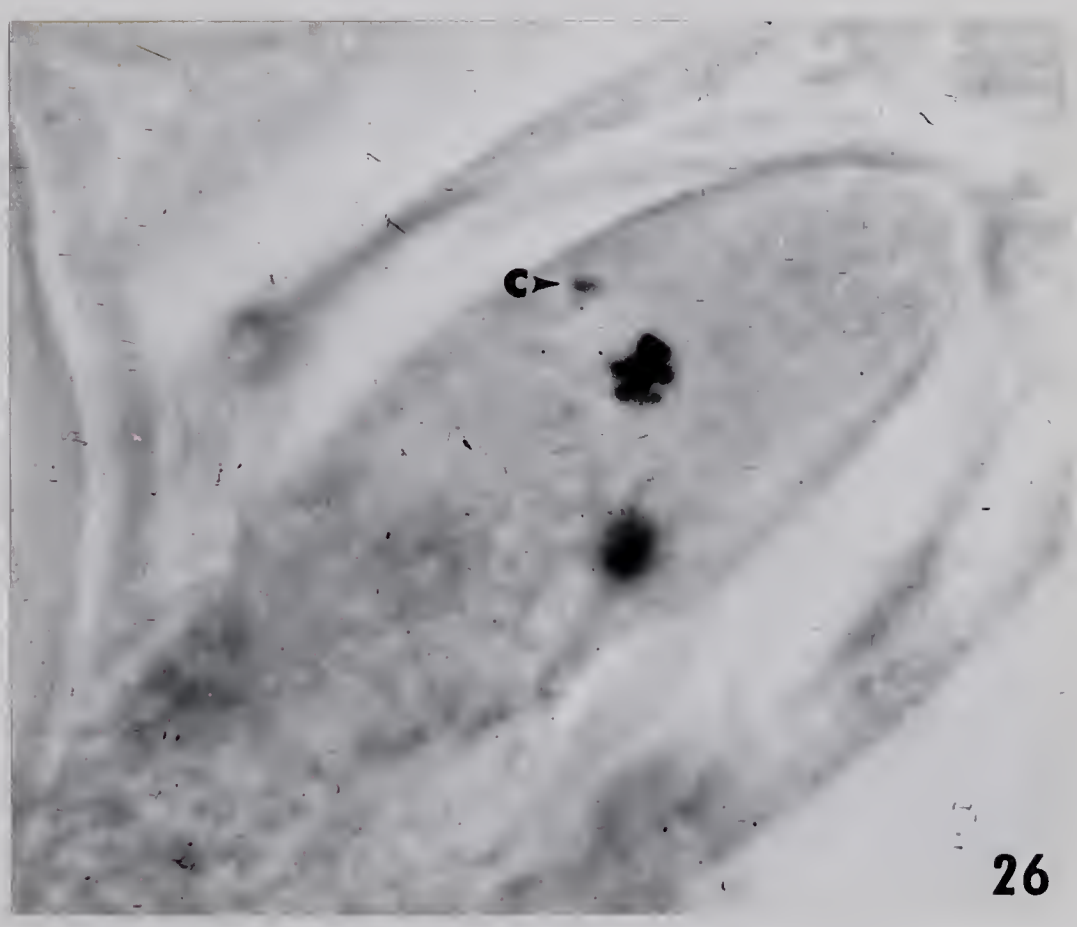

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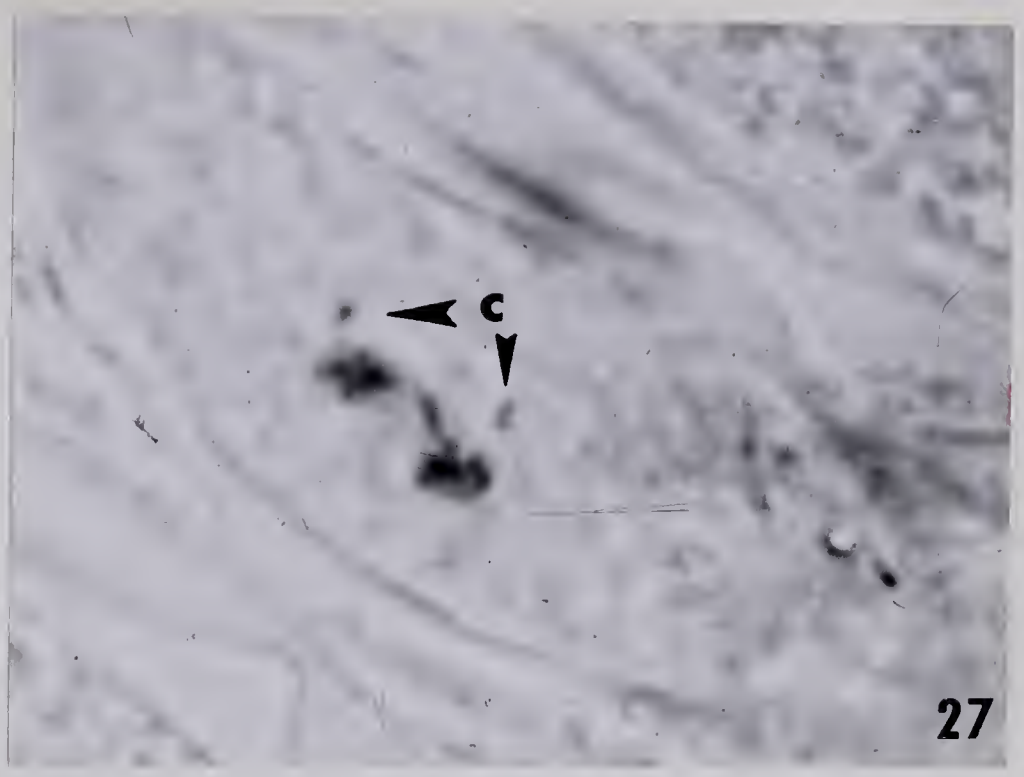
Fig. 27. Late telophase - early interphase of Division IV of ascosporogenesis. Two globular centrioles are visible. Feulgen staining. 2200X.

Fig. 28. Late telophase - early interphase of Division IV of ascosporogenesis. Two globular centrioles are visible. Feulgen staining. 2200X.

Fig. 29. Late telophase - early interphase of Division IV of ascosporogenesis. Two globular centrioles are visible. Feulgen staining. 2200X.

c = centriole



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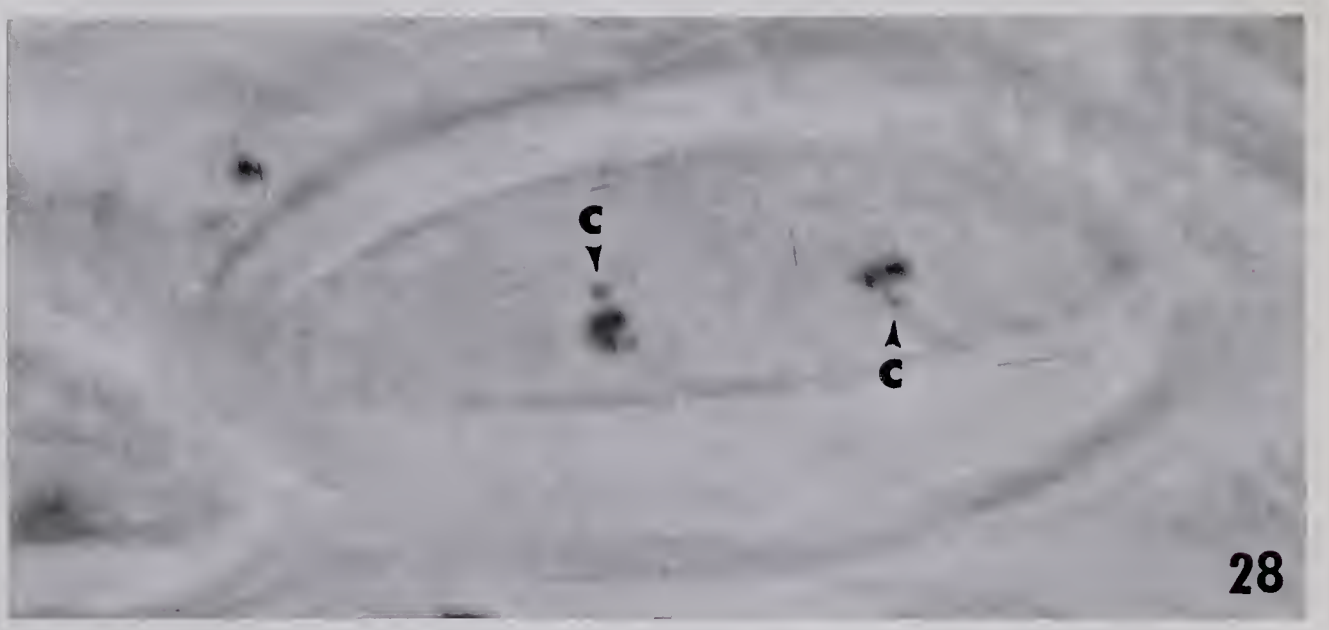

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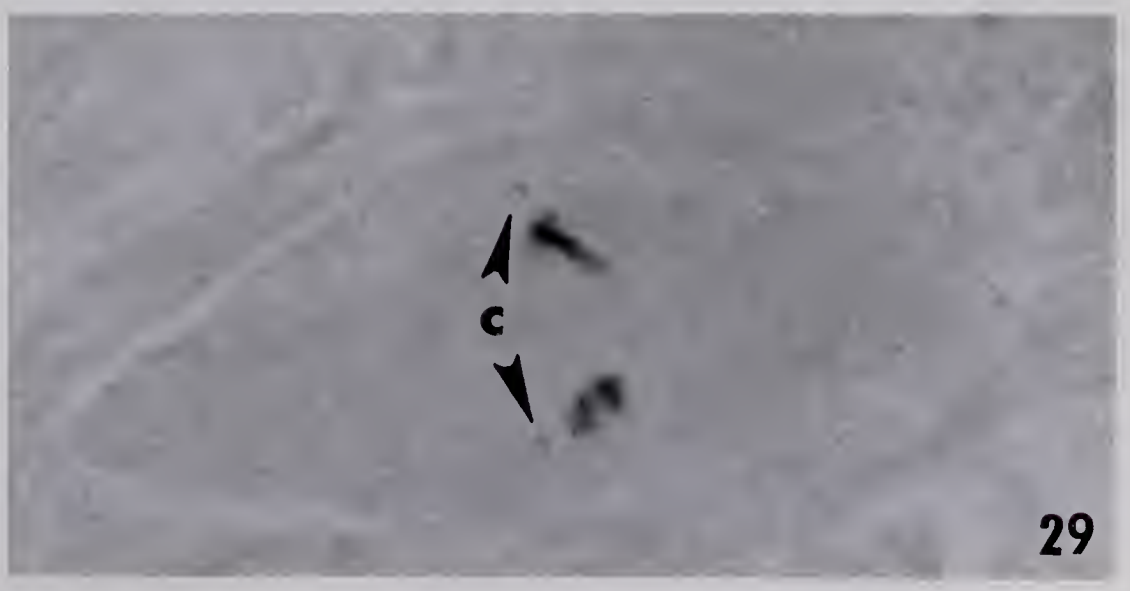

29

Fig. 30.*

A.- Primary nucleus with extranuclear bodies. Cytoplasm differentiated into the central spore plasm, and epiplasm at each end with large vacuoles. Shrinkage during fixation at the upper and lower ends.
B.- Metaphase of the first division. The spindle is parallel to the long axis of the ascus. The pointed ends of the spindle take the safranin stain readily.
C.- One type of the two-nucleate stage where the nuclei are not oriented on the central longitudinal axis of the ascus; large vacuoles in either end of the ascus.
D.- Second division in metaphase stages. The spindles are longitudinal, one somewhat above the other but at a different focus.
E.- Another type of division in which the spindles lie oblique to walls of the ascus. The remains of the old nuclear membrane somewhat collapsed, show distinctly on both of the spindles. Very little cytoplasm in either end of the ascus; spore plasm rather dense and finely granular.
F.- Late telophase of the second division. Spindles oblique, one somewhat above and parallel to the other. The polar chromatin masses connected by filamentous structures which take the stain rather heavily. Remains of the parent nuclear material seen at the center of the upper spindle.
G.- Four-nucleate stage, the pair of nuclei lying at higher focus, more heavily shaded. Such a distribution of the nuclei may well result from divisions similar to those shown in Figures E and F. The two upper nuclei are somewhat pear shaped. The relationship of the nuclei can be determined by the location of the central bodies; compare with I.
H.- Two views of the same nucleus somewhat more enlarged than in other figures. At the left and seen at high focus, the nucleus appears nearly spherical or without particular distortion. At the right, part of the same nucleus, seen at a lower focus, now shows the beak-like cap with forked appendages.
I.- Four-nucleate stage in resting condition. Nucleoles distinct. Each nucleus is pear shaped or crowned by a beak-like cap of fibers at the end of which is a forked appendage. The beaks of the sister nuclei extend in opposite directions. The spore plasm at this stage is divided into two parts by a series of central vauoles.
J and K.- Two sections of the same ascus during metaphase stages of the third division. J should have been mounted so that the nucleus would have been at about the same level as the lower nucleus in K. At the upper end of the ascus in K can be seen one complete spindle and the tip end of a second, the remainder of which appeared in the next section and was not drawn.

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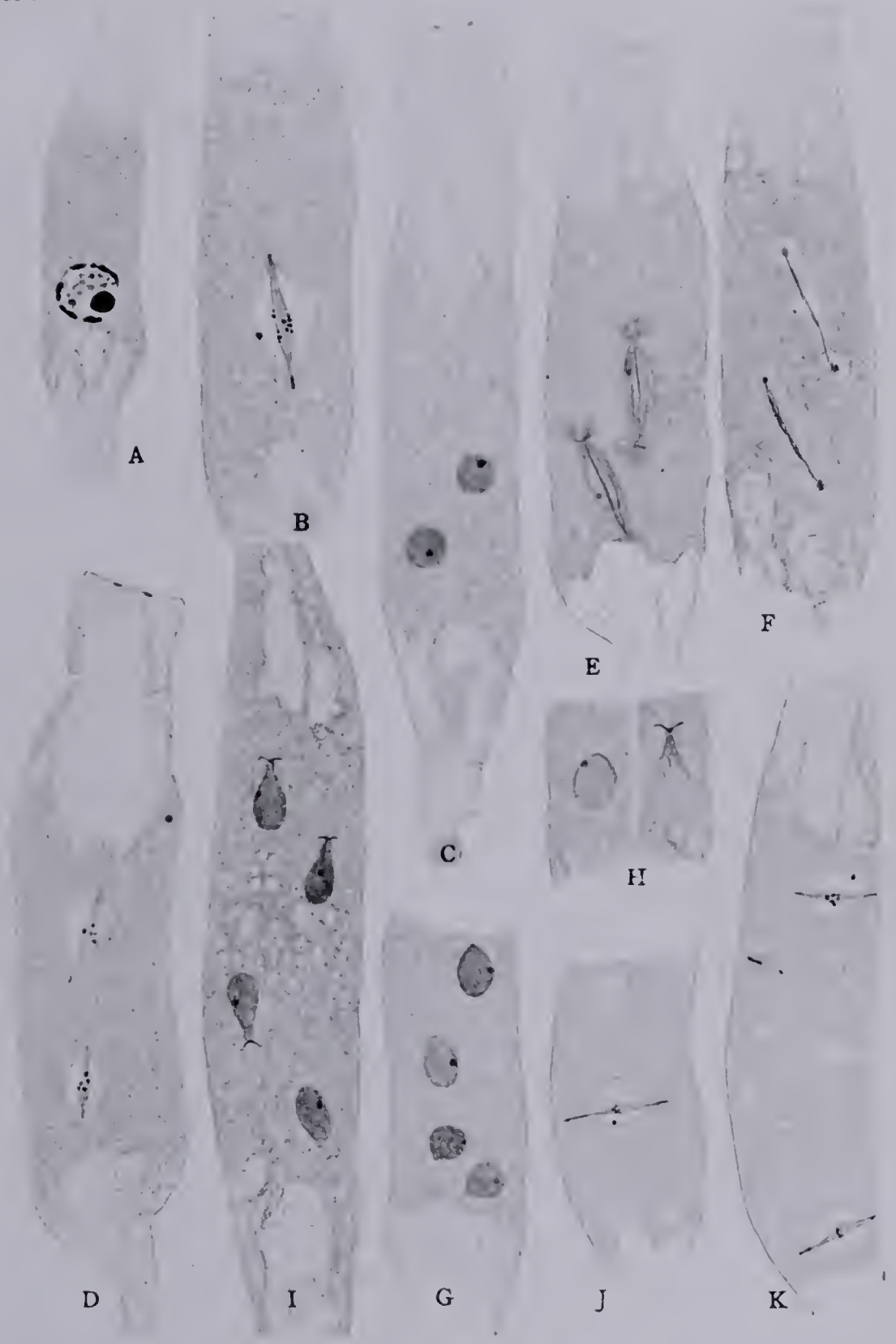
* Taken from Dodge (15).



(For explanatory legend see p. 290)